Current Topics in Behavioral Neurosciences 13

Wolfgang H. Sommer
Rainer Spanagel *Editors*

Behavioral Neurobiology of Alcohol Addiction

Current Topics in Behavioral Neurosciences

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Wolfgang H. Sommer
Rainer Spanagel
Editors

Behavioral Neurobiology of Alcohol Addiction

Editors
Dr. Wolfgang H. Sommer
Institut für Psychopharmakologie
Zentralinstitut für Seelische Gesundheit
Mannheim
Germany

Prof. Dr. Rainer Spanagel
Institut für Psychopharmakologie
Zentralinstitut für Seelische Gesundheit
Mannheim
Germany

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Preface

Dear Reader

You might think that in the age of internet and PubMed, books on current topics are outdated already ahead of publication, or that books are too sluggish in the rapid currents of information flows. At first, so did we, yet after a moment's notice one realizes that books, better than most electronic media, can provide highly needed anchoring points for looking around, taking stock, and contemplation on present scientific endeavors, and on which new direction to take. Reflecting on Current Topics of Behavioral Neurobiology in Alcohol Addiction was for us an orienting response, an opportunity to see our field through the eyes of many of our most esteemed colleagues and a way to engage them in discussion how alcohol can alter mood states and why this may end up becoming an addiction. With this book we hope to share ours and our contributor's excitement about the subject matter with a broad readership. Indeed, today's alcohol research generates more excitement than ever. This is visibly demonstrated by a more than threefold increase in the number of articles published in the top-notch journals of the general and multidisciplinary science category over the last decade (Helinski and Spanagel 2011) . Naturally, the greater attention created by our field attracts new generations of students. Thus, besides contributing a comprehensive collection of reference material accompanied by critical discussions for the seasoned scholars, Current Topics in Behavioral Neurobiology of Alcohol Addiction aims towards new disciples in addiction research as well as interested readers from other fields of study by providing lucid presentations of these topics that are written by an assembly of highly distinguished experts and leaders in their respective research areas.

Alcohol addiction research will ultimately be judged by its ability to provide effective treatment solutions. To be successful here requires cunning and understanding far beyond Behavioral Neurobiologyand other disciplines of the neurosciences including basic and clinical research, but also genetics, epidemiology, social sciences and computational approaches. Such diversity is bound to generate a constant stream of new observations and ideas that want to be pursued. Thus, our most difficult task as editors was to refrain from reviewing these latest

developments, but to select those Current Topics that have brought alcohol addiction research substantially forward and strongly influenced the thinking about it. With this idea in mind we selected five Current Topics in Behavioral Neurobiology of Alcohol Addiction which are discussed in their respective parts of the book.

The part I deals with the conceptualization of addiction and underlying neurobiological mechanisms. This topic is important because there is an ongoing debate about the role of the mesolimbic dopamine system in driving and maintaining addictive behaviors, especially for alcohol addiction. Further, exciting new concepts such as the glutamate theory of addiction or the importance of anti-reward systems have emerged and driven new investigation into the cellular and synaptic consequence of alcohol exposure.

The part II takes up genetic approaches which in the last decade have enormously influenced psychiatric research. Notable, with the early accomplishment of genome-wide association studies (GWAS) and the emergence of candidate genes derived from cohorts of tens of thousands of subjects the alcohol field has been in the forefront of psychiatric genetics. Here, the new insights from genome-wide studies in humans and experimental animals are discussed in view of their ramifications for understanding alcohol use disorder as a diagnostic entity in current systems of psychiatric diagnoses. Chapters on the influence of genetic factors on alcohol behaviors in non-human species supplement this part. Given the ever more sophisticated techniques for genetic manipulations and the large number of genes that may influence alcohol behaviors we found it justified to invite a new review on genetically modified mouse models, the latest being completed already six years ago[2].

The part III takes a look at the broad range of procedures for testing new and existing hypotheses about addictive behaviors in appropriate animal experiments and more recently in the human laboratory. Concerning animal models there is an apparent shift towards much longer duration of alcohol exposure reflecting the increased emphasis on the chronic progressive course of addiction and the drive to discover pathology-related long-term neuroadaptations underlying it.

Another area of great development is neuroimaging which is considered in part IV of this volume. Although a relatively novel tool for studying the human brain, we may already conclude that many human neuroimaging experiments recapitulate our knowledge from animal studies about the neurocircuitry involved in the action of alcohol and addiction, and in this way are giving much needed validity to our animal models. Imaging responses specific to the human disorder or translatable between humans and animals may hold promises for identification of easily accessible biomarkers for treatment development.

The part V of the book is dedicated to translational approaches for treatment development in alcohol addiction. Although many researchers in the field may feel that they always had a translational perspective to the subject matter, the last decade has truly put the focus on the need of translating knowledge gained from basic results more rapidly into clinical developments. The term "translational research" is in our mind not a mere buzz-word, rather the stringent application of

this concept has truly brought our field forward. Although, no new therapeutics could be introduced into clinical practice in recent years, the examples presented here, ranging from psychotherapy to pharmacology to neurosurgery, demonstrate the power of the translational approach and raise hopes that the dire situation of alcoholic patients can be changed in the near future.

Finally, we deeply thank all our contributors for the enthusiasm, dedication and patience they have invested into this project and into us, because working for a book like the present one can only be seen as an act of great passion for science. We are humbled by their altruism and collegiality evident by the near lack of any reward expectation, neither monetary nor impact points wise, to offset the time and trouble that undeniably has to be laid down into such an endeavor. We are grateful to the Springer team for their encouragement, help and endurance in publishing this book.

Mannheim, Germany

Wolfgang Sommer
Rainer Spanagel

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Contents

Part I Mechanistic Aspects Underlying Alcoholism

Theoretical Frameworks and Mechanistic Aspects of Alcohol Addiction: Alcohol Addiction as a Reward Deficit Disorder 3
George F. Koob

Synaptic Effects Induced by Alcohol 31
David M. Lovinger and Marisa Roberto

Signaling Pathways Mediating Alcohol Effects 87
Dorit Ron and Robert O. Messing

Neurocircuitry Involved in the Development of Alcohol Addiction: The Dopamine System and its Access Points. 127
Bo Söderpalm and Mia Ericson

What is in that Drink: The Biological Actions of Ethanol, Acetaldehyde, and Salsolinol 163
Gerald A. Deehan Jr., Mark S. Brodie and Zachary A. Rodd

Part II Genetics

Modeling the Diagnostic Criteria for Alcohol Dependence with Genetic Animal Models 187
John C. Crabbe, Kenneth S. Kendler and Robert J. Hitzemann

Non-Human Primate Models of Alcohol-Related Phenotypes: The Influence of Genetic and Environmental Factors 223
Christina S. Barr

Genetically Selected Alcohol Preferring Rats to Model Human Alcoholism 251
Roberto Ciccocioppo

Advanced Transgenic Approaches to Understand Alcohol-Related Phenotypes in Animals 271
Ainhoa Bilbao

Part III Clinical Phenotypes and Preclinical Models Thereof

Modeling Alcohol Self-Administration in the Human Laboratory 315
Ulrich S. Zimmermann, Sean O'Connor and Vijay A. Ramchandani

Animal Models of Excessive Alcohol Consumption in Rodents 355
Howard C. Becker

Modeling Relapse Situations in the Human Laboratory 379
Rajita Sinha

Modeling Relapse in Animals 403
Rémi Martin-Fardon and Friedbert Weiss

Invertebrate Models of Alcoholism 433
Henrike Scholz and Julie A. Mustard

Part IV Novel Insights from Brain Imaging

The Dopamine System in Mediating Alcohol Effects in Humans 461
K. Charlet, A. Beck and A. Heinz

Stimulant and Sedative Effects of Alcohol 489
Reuben A. Hendler, Vijay A. Ramchandani, Jodi Gilman and Daniel W. Hommer

Chronic Alcohol Consumption, Abstinence and Relapse: Brain Proton Magnetic Resonance Spectroscopy Studies in Animals and Humans 511
Dieter J. Meyerhoff, Timothy C. Durazzo and Gabriele Ende

Part V Translational Aspects and Medication Development

Translational Approaches to Medication Development 543
Selena Bartlett and Markus Heilig

New Pharmacological Treatment Strategies for Relapse Prevention 583
Rainer Spanagel and Valentina Vengeliene

The Challenge of Studying Parallel Behaviors in Humans and Animal Models 611
David N. Stephens, Hans S. Crombag and Theodora Duka

A Translational Approach to Novel Medication Development for Protracted Abstinence 647
Barbara J. Mason and Amanda E. Higley

New Approaches to Addiction Treatment Based on Learning and Memory 671
Falk Kiefer and Christina Dinter

Adolescent Substance Misuse: Neurobiology and Evidence-Based Interventions 685
Nicola C. Newton, Maeve O'Leary-Barrett and Patricia J. Conrod

Deep Brain Stimulation as a Therapy for Alcohol Addiction 709
Thomas F. Münte, Hans-Jochen Heinze and Veerle Visser-Vandewalle

Part I
Mechanistic Aspects Underlying Alcoholism

Theoretical Frameworks and Mechanistic Aspects of Alcohol Addiction: Alcohol Addiction as a Reward Deficit Disorder

George F. Koob

Abstract Alcoholism can be defined by a compulsion to seek and take drug, loss of control in limiting intake, and the emergence of a negative emotional state when access to the drug is prevented. Alcoholism impacts multiple motivational mechanisms and can be conceptualized as a disorder that includes a progression from impulsivity (positive reinforcement) to compulsivity (negative reinforcement). The compulsive drug seeking associated with alcoholism can be derived from multiple neuroadaptations, but the thesis argued here is that a key component involves the construct of negative reinforcement. Negative reinforcement is defined as drug taking that alleviates a negative emotional state. The negative emotional state that drives such negative reinforcement is hypothesized to derive from dysregulation of specific neurochemical elements involved in reward and stress within the basal forebrain structures involving the ventral striatum and extended amygdala, respectively. Specific neurochemical elements in these structures include not only decreases in reward neurotransmission, such as decreased dopamine and γ-aminobutyric acid function in the ventral striatum, but also recruitment of brain stress systems, such as corticotropin-releasing factor (CRF), in the extended amygdala. Acute withdrawal from chronic alcohol, sufficient to produce dependence, increases reward thresholds, increases anxiety-like responses, decreases dopamine system function, and increases extracellular levels of CRF in the central nucleus of the amygdala. CRF receptor antagonists also block excessive drug intake produced by dependence. A brain stress response system is hypothesized to be activated by acute excessive drug intake, to be sensitized during repeated withdrawal, to persist into protracted abstinence, and to contribute to the compulsivity of alcoholism. Other components of brain stress

G. F. Koob (✉)
Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2400, La Jolla, CA 92037, USA
e-mail: gkoob@scripps.edu

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systems in the extended amygdala that interact with CRF and that may contribute to the negative motivational state of withdrawal include norepinephrine, dynorphin, and neuropeptide Y. The combination of loss of reward function and recruitment of brain stress systems provides a powerful neurochemical basis for a negative emotional state that is responsible for the negative reinforcement driving, at least partially, the compulsivity of alcoholism.

Keywords Addiction · Opponent process · Stress · Extended amygdala · Corticotropin-releasing factor

Contents

1 Definitions and Conceptual Framework for Reward Deficit in Alcoholism 4
1.1 Theoretical Framework: Motivation, Withdrawal, and Opponent Process 8
2 Animal Models for Compulsive Alcohol Seeking 12
3 Neural Substrates for the Negative Emotional State Associated with Alcoholism 13
3.1 Within-System Neuroadaptations that Contribute to the Compulsivity Associated with the Dark Side of Alcoholism 13
3.2 Between-System Neuroadaptations that Contribute to Compulsivity Associated with the Dark Side of Alcoholism 16
4 Compulsivity in Alcoholism: an Allostatic View 19
References 23

1 Definitions and Conceptual Framework for Reward Deficit in Alcoholism

Alcoholism has many definitions that vary from social frameworks to a psychiatric framework embedded in the diagnosis of Substance Dependence on Alcohol defined in the *Diagnostic and Statistical Manual of the American Psychiatric Association*, 4th edition (DSM-IV; American Psychiatric Association 1994). Alcoholism, and more generically drug addiction, can be defined as a chronically relapsing disorder characterized by (i) compulsion to seek and take the drug (alcohol), (ii) loss of control in limiting (alcohol) intake, and (iii) emergence of a negative emotional state (e.g., dysphoria, anxiety and irritability) reflecting a motivational withdrawal syndrome when access to the drug (alcohol) is prevented (defined here as dependence; Koob and Le Moal 1997). Clinically and in animal models, the occasional but limited use of alcohol with the *potential* for abuse or dependence is distinct from escalated alcohol intake and the emergence of a chronic alcohol-dependent state. The thesis argued in the present synthesis is that alcoholism, similar to drug addiction, is a reward deficit disorder, and the "emergence of a negative emotional state" plays an important role in defining and

perpetuating alcoholism. Alcoholism also involves substantial neuroadaptations that persist beyond acute withdrawal and trigger relapse and deficits in cognitive function that can also fuel compulsive drinking. However, the argument here is that the core deficit that sets up vulnerability to relapse in alcoholism, and possibly even deficits in cognitive function, is in fact decreased reward function.

To support this hypothesis, a holistic view of alcoholism will be presented with the following arguments. A negative emotional state is a common presentation in most alcoholics during withdrawal and protracted abstinence. Compulsivity observed in alcoholism has an important negative reinforcement component that perpetuates alcoholism. Such negative emotional states become sensitized over time and set up an allostatic state that perpetuates dependence. Negative emotional states set up a powerful motivational state for relapse. Finally, the neurobiological substrates underlying the motivation to seek alcohol will be reviewed, and an argument will be presented that it is loss of reward function and gain of brain stress function that mediate the negative emotional state outlined as key to alcoholism.

Drug addiction has generally been conceptualized as a disorder that involves elements of both impulsivity and compulsivity, in which *impulsivity* can be defined behaviorally as "a predisposition toward rapid, unplanned reactions to internal and external stimuli without regard for the negative consequences of these reactions to themselves or others" (Moeller et al. 2001). Impulsivity is measured in two domains: the choice of a smaller, immediate reward over a larger, delayed reward (Rachlin and Green 1972) or the inability to inhibit behavior by changing the course of action or to stop a response once it is initiated (Logan et al. 1997). Impulsivity is a core deficit in substance abuse disorders (Allen et al. 1998) and neuropsychiatric disorders such as attention deficit hyperactivity disorder. Operationally, delay-to-gratification tasks (delayed discounting tasks; impulsive choice) and the stop-signal or go/no-go task (behavioral impulsivity) have been used as measures of impulsivity (Fillmore and Rush 2002; Green et al. 1994). *Compulsivity* can be defined as elements of behavior that result in perseveration of responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations (e.g., operationally, responding for a drug or alcohol in the face of adverse consequences (Wolffgramm and Heyne 1995) or responding for a drug or alcohol on a progressive-ratio schedule of reinforcement (Walker et al. 2008)). Compulsivity is analogous to the symptoms of Substance Dependence outlined by the American Psychiatric Association: continued substance use despite knowledge of having had a persistent or recurrent physical or psychological problem and a great deal of time spent in activities necessary to obtain the substance (American Psychiatric Association 2000).

Collapsing the cycles of impulsivity and compulsivity yields a composite addiction cycle comprising three stages—*preoccupation/anticipation*, *binge/intoxication*, and *withdrawal/negative affect*—in which impulsivity often dominates at the early stages and compulsivity dominates at terminal stages (Fig. 1). As an individual moves from impulsivity to compulsivity, a shift occurs from positive reinforcement driving the motivated behavior to negative reinforcement driving the motivated behavior (Koob 2004). Negative reinforcement can be defined as the

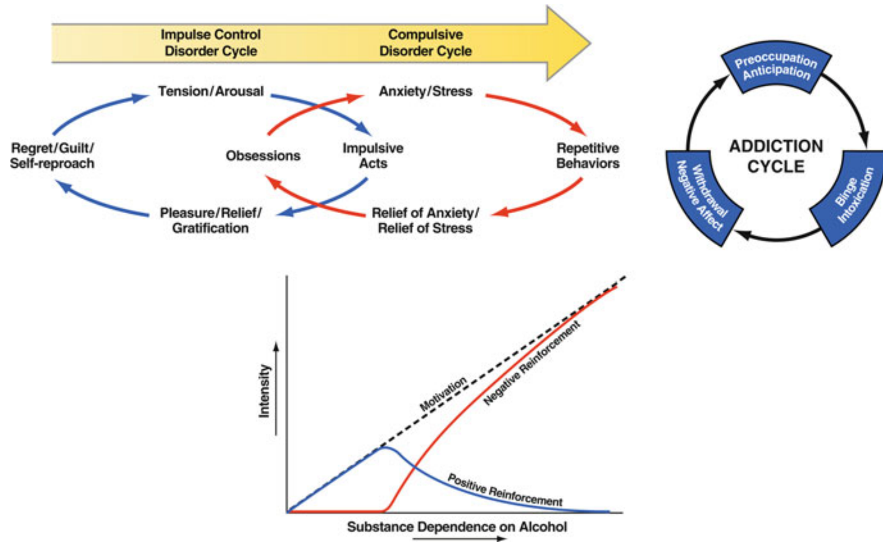

Fig. 1 (*Top left*) Diagram showing the stages of impulse control disorder and compulsive disorder cycles related to the sources of reinforcement. In impulse control disorders, an increasing tension and arousal occurs before the impulsive act, with pleasure, gratification, or relief during the act. Following the act, there may or may not be regret or guilt. In compulsive disorders, there are recurrent and persistent thoughts (obsessions) that cause marked anxiety and stress followed by repetitive behaviors (compulsions) that are aimed at preventing or reducing distress (American Psychiatric Association 1994). Positive reinforcement (pleasure/gratification) is more closely associated with impulse control disorders. Negative reinforcement (relief of anxiety or relief of stress) is more closely associated with compulsive disorders. (*Top right*) Collapsing the cycles of impulsivity and compulsivity results in the addiction cycle, conceptualized as three major components: *preoccupation/anticipation*, *binge/intoxication*, and *withdrawal/negative affect* (Taken with permission from Koob 2008b.) (*Bottom*) Change in the relative contribution of positive and negative reinforcement constructs during the development of substance dependence on alcohol

process by which removal of an aversive stimulus (e.g., negative emotional state of drug withdrawal) increases the probability of a response (e.g., dependence-induced drug intake to relieve the negative emotional state). Note that negative reinforcement is not punishment, although both involve an aversive stimulus. In punishment, the aversive stimulus suppresses behavior, including drug taking (e.g., disulfiram [Antabuse]). Negative reinforcement can be perhaps described in lay terms as reward via relief (i.e., relief reward), such as removal of pain or in the case of alcoholism removal of the negative emotional state of acute withdrawal or protracted abstinence. The three stages are conceptualized as interacting with each other, becoming more intense, and ultimately leading to the pathological state known as addiction (Koob and Le Moal 1997) (Fig. 1).

In alcohol addiction, or alcoholism, a pattern of oral drug taking evolves that is often characterized by binges of alcohol intake that can be daily episodes or prolonged days of heavy drinking and is characterized by a severe emotional and

somatic withdrawal syndrome. Many alcoholics continue with such a binge/withdrawal pattern for extended periods of time, but some individuals can evolve into an opioid-like situation in which they must have alcohol available at all times to avoid the negative consequences of abstinence. Here, intense preoccupation with obtaining alcohol (craving) develops that is linked not only to stimuli associated with obtaining the drug but also to stimuli associated with withdrawal and the aversive motivational state. A pattern develops in which the drug must be obtained to avoid the severe dysphoria and discomfort of abstinence.

The pattern of alcohol addiction, related to reward dysfunction, can be amply illustrated by excerpts from two case histories from Knapp (1996) and Goodwin (1981). In the first representative case history, an individual progresses from the state where they stated, "I drank when I was happy and I drank when I was anxious and I drank when I was bored and I drank when I was depressed, which was often," to, "I loved the way drink made me feel, and I loved its special power of deflection, its ability to shift my focus away from my own awareness of self and onto something else, something less painful than my own feelings," and, "There's a sense of deep need, and the response is a grabbiness, a compulsion to latch on to something outside yourself in order to assuage some deep discomfort" (Knapp 1996). Similarly, in a second representative case history, "Alcohol seemed to satisfy some specific need I had, which I can't describe," and, "There were always reasons to drink. I was low, tense, tired, mad, happy," and, "The goal, always, was to maintain a glow, not enough, I hoped, that people would notice, but a glow," and, "By now I was hooked and knew it, but desperately did not want others to know it. I had been sneaking drinks for years—slipping out to the kitchen during parties and such—but now I began hiding alcohol, in my desk, bedroom, car glove compartment, so it would never be far away, ever. I grew panicky even thinking I might not have alcohol when I needed it, which was just about always," and, "I loathed myself. I was waking early and thinking what a mess I was, how I had hurt so many others and myself. The words 'guilty' and 'depression' sound superficial in trying to describe how I felt. The loathing was almost physical—a dead weight that could be lifted in only one way, and that was by having a drink"(Goodwin 1981; see Koob and Le Moal 2006, Appendix, for full quotations).

These case histories illustrate numerous key points regarding the present treatise, but the main point to be further discussed below is the transition from drinking to feel good to drinking to avoid feeling bad. To some extent, this transition is facilitated by personality differences, presumably shaped not only by genetics but also by developmental and even social factors. As Khantzian (1997) cogently argued, addiction can be considered a type of chronic emotional distress syndrome that varies with the individual from physical and emotional pain to chronic dysphoria to stress and anxiety to interpersonal difficulties for which drugs can be argued to be sources of self-medication for such negative emotional states. Additionally, he argued that self-medication may be drug-specific—patients may have a preferential use of drugs that fits with the nature of the painful feeling states that they are self-medicating (e.g., opiates to counter intense anger and rage,

stimulants as augmenting agents for high-energy individuals, energizing agents for low-energy individuals, and depressants [e.g., alcohol] for individuals who are tense and anxious). The common element argued by Khantzian is that each class of drugs serves as antidotes or correctives to dysphoric states and acts as a "replacement for a defect in the psychological structure" (Kohut 1971, p. 46) of such individuals (Khantzian 2003).

1.1 Theoretical Framework: Motivation, Withdrawal, and Opponent Process

Motivation is a state that can be defined as a "tendency of the whole animal to produce organized activity" (Hebb 1972), and such motivational states are not constant but rather vary over time. Early work by Wikler stressed the role of changes in drive states associated with dependence. Subjects described changes in withdrawal as a "hunger" or primary need and the effects of morphine on such a state as "satiation" or gratification of the primary need (Wikler 1952). Although Wikler argued that positive reinforcement was retained even in heavily dependent subjects (thrill of the intravenous opioid injection), dependence produced a new source of gratification, that of negative reinforcement (see above).

The concept of motivation in addiction was inextricably linked with hedonic, affective, or emotional states in the context of temporal dynamics by Solomon's opponent process theory of motivation. Solomon and Corbit (1974) postulated that hedonic, affective, or emotional states, once initiated by drugs, are automatically modulated by the central nervous system with mechanisms that reduce the intensity of hedonic feelings. The *a-process* includes affective or hedonic habituation (or tolerance), and the *b-process* includes affective or hedonic withdrawal (abstinence). The *a-process* in drug use consists of positive hedonic responses, occurs shortly after presentation of a stimulus, correlates closely with the intensity, quality, and duration of the reinforcer, and shows tolerance. In contrast, the *b-process* in drug use appears after the *a-process* has terminated, consists of negative hedonic responses, and is sluggish in onset, slow to build up to an asymptote, slow to decay, and gets larger with repeated exposure. The thesis here is that opponent processes begin early in drug taking, reflect changes in the brain reward and stress systems, and later form one of the major motivations for compulsivity in drug taking.

Thus, dependence or manifestation of a withdrawal syndrome after removal of chronic drug administration is defined in terms of *motivational* aspects of dependence, such as emergence of a negative emotional state (e.g., dysphoria, anxiety and irritability) when access to the drug is prevented (Koob and Le Moal 2001), rather than on the *physical* signs of dependence. Indeed, some have argued that the development of such a negative affective state can define dependence as it relates to addiction:

> The notion of dependence on a drug, object, role, activity or any other stimulus-source requires the crucial feature of negative affect experienced in its absence. The degree of dependence can be equated with the amount of this negative affect, which may range from mild discomfort to extreme distress, or it may be equated with the amount of difficulty or effort required to do without the drug, object, etc (Russell 1976).

Alcoholics show dramatic evidence of dysphoric states during acute withdrawal that persist into protracted abstinence. Alcohol withdrawal in humans produces well documented physical (somatic) symptoms, such as tremor, autonomic hyperactivity, nausea, vomiting, and seizures, but more importantly produces significant affective symptoms of anxiety, dysphoria, and depression-like symptoms. Acute withdrawal (i.e., the first week post-alcohol) is characterized by Beck Depression Inventory scores of approximately 20, which is categorized within the range of moderate depression (Potokar et al. 1997; 15–30), and Hamilton Depression Scores of 18, which is close to 20 (the cutoff for antidepressant medication in affective disorder; Brown and Schuckit 1988). Depression scores decline during subsequent weeks of treatment but remain at close to 10 for Hamilton Depression Scores for up to 4 weeks of an inpatient treatment program (Brown and Schuckit 1988). In another study of inpatient alcoholics during withdrawal, the Beck Depression Inventory score was at 15 at withdrawal and remained at 12.8 two days into withdrawal and at 9.4 two weeks post-withdrawal (de Timary et al. 2008). Similar results were obtained for anxiety measures (Potokar et al. 1997; de Timary et al. 2008). In another study with a long-term follow-up of 6 months after a 4-week inpatient detoxification, Beck Depression Inventory scores remained at approximately 6, and trait anxiety scores (STAI-X2) remained above 33 even in subjects without comorbid anxiety or depression (Driessen et al. 2001). Independent of comorbidity status, individuals who relapsed had higher trait anxiety scores than those who abstained (Driessen et al. 2001). Thus, although alcoholics show significant decreases in measures of depression and anxiety during withdrawal, there is a measurable level of depression-like symptoms that persist long after acute withdrawal into protracted abstinence that may be clinically (treatment) relevant.

More compelling for the present thesis, during a 2-week inpatient withdrawal study, alexithymia (defined as a state of deficiency in understanding, processing, or describing emotions; from the Greek *a* for "lack," *lexis* for "word," and *thymos* for "emotion"; Sifneos 1973; Taylor and Bagby 2000), which results in poor emotional regulation and stress management abilities, remained high and stable during the 2-week period (de Timary et al. 2008). Alexithymia scores did not decline between the 0 and 2 day time-points but remained high at a score of 57 and declined only to 53 at the 3-week time-point (de Timary et al. 2008). The authors argued that alexithymia is a stable personality trait in alcoholics rather than a state-dependent phenomenon, providing support for the self-medication hypothesis outlined above.

Animal models can also be used to test the hypothesis that there are opponent process-like motivational changes associated with the development of alcohol dependence. Electrical brain stimulation reward or intracranial self-stimulation has a long history as a measure of activity of the brain reward system and of the acute reinforcing effects of drugs of abuse. All drugs of abuse, when administered acutely, decrease brain stimulation reward thresholds (Kornetsky and Esposito 1979) and

when administered chronically increase reward thresholds during withdrawal (see above). Brain stimulation reward involves widespread neurocircuitry in the brain, but the most sensitive sites defined by the lowest thresholds involve the trajectory of the medial forebrain bundle that connects the ventral tegmental area with the basal forebrain (Olds and Milner 1954; Koob et al. 1977). Although much emphasis was focussed initially on the role of the ascending monoamine systems in the medial forebrain bundle in brain stimulation reward, other nondopaminergic systems in the medial forebrain bundle clearly play a key role (Hernandez et al. 2006).

Rats made dependent using chronic ethanol vapor exposure at blood alcohol levels sufficient to drive excessive drinking showed an increase in brain reward thresholds during withdrawal that lasted up to 3 days post-withdrawal (Schulteis et al. 1995). However, data suggest that, similar to other drugs of abuse, such opponent-like processes can begin with a single dosing (Fig. 2).

An acute elevation in brain reward thresholds was observed during repeated acute withdrawal from ethanol, bearing a striking resemblance to human subjective reports (Schulteis and Liu 2006) (Fig. 2). These results demonstrate that the elevation in brain reward thresholds following prolonged access to alcohol may fail to return to baseline levels between repeated and prolonged exposure to alcohol self-administration (i.e., a residual reward deficit), thus creating the greater elevation in reward thresholds observed during withdrawal from chronic ethanol. Rapid acute tolerance and opponent process-like effects in response to the hedonic effects of alcohol have been reported in human studies using the alcohol clamp procedure (Morzorati et al. 2002). These data provide compelling evidence for brain reward dysfunction with chronic alcohol, which provides strong support for a hedonic allostasis model of alcoholism (Koob 2003).

The dysregulation of brain reward function associated with withdrawal from chronic administration of drugs of abuse is a common element of all drugs of abuse. Withdrawal from chronic cocaine (Markou and Koob 1991), amphetamine (Paterson et al. 2000), opioids (Schulteis et al. 1994), cannabinoids (Gardner and Vorel 1998), nicotine (Epping-Jordan et al. 1998), and ethanol (Schulteis et al. 1995) leads to increases in reward thresholds during acute abstinence, and some of these elevations in threshold can last for up to 1 week. These observations lend credence to the hypothesis that opponent processes can set the stage for one aspect of compulsivity in which negative reinforcement mechanisms are engaged.

More recently, the opponent process theory has been expanded into the domains of the neurobiology of drug addiction from a neurocircuitry perspective. An allostatic model of the brain motivational systems has been proposed to explain the persistent changes in motivation that are associated with dependence in addiction (Koob and Le Moal 2001, 2008). In this formulation, addiction is conceptualized as a cycle of increasing dysregulation of brain reward/anti-reward mechanisms that results in a negative emotional state contributing to the compulsive use of drugs. Counter-adaptive processes that are part of the normal homeostatic limitation of reward function fail to return within the normal homeostatic range. These counteradaptive processes are hypothesized to be mediated by two mechanisms: within-system neuroadaptations and between-system neuroadaptations (Koob and Bloom 1988).

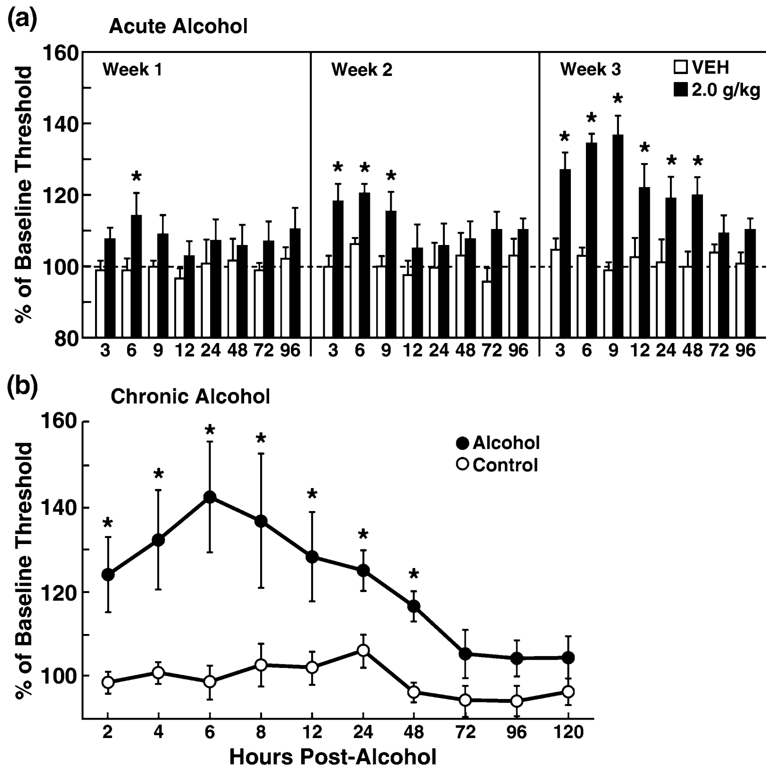

Fig. 2 **a** Withdrawal from a single bout of acute ethanol intoxication (week 1) resulted in a significant but transient increase in brain reward threshold only with the highest dose of ethanol tested (2.0 g/kg; $^{a}P < 0.05$, compared with vehicle controls at given time-point post-injection). The effect was significant at 6 hours, a time when blood alcohol levels had declined to virtually undetectable levels following this dose of ethanol. Repeated treatment with this dose for two additional weeks resulted in a progressive broadening of the duration of significant threshold elevations. By comparison, treatment with 1.5 g/kg ethanol resulted in significant but transient elevations only after three repeated bouts of intoxication/withdrawal, and no statistically reliable changes were seen after one or two treatments (data not shown). Treatment with 1.0 g/kg did not produce any statistically reliable threshold changes regardless of treatment week (data not shown). Data are expressed as mean ± SEM percentage of baseline threshold. $n = 8$–10 per dose group. [Taken with permission from Schulteis and Liu 2006.] **b** Time-dependent elevation of intracranial self-stimulation thresholds during ethanol withdrawal. Mean blood alcohol levels were 197.29 mg%. Data are expressed as mean ± SEM percentage of baseline threshold. $^{a}p < 0.05$, thresholds that were significantly elevated above control levels at 2–48 hours post-ethanol. Open circles indicate the control condition. Closed circles indicate the ethanol withdrawal condition. [Taken with permission from Schulteis et al. 1995.]

In a within-system neuroadaptation, "the primary cellular response element to the drug would itself adapt to neutralize the drug's effects; persistence of the opposing effects after the drug disappears would produce the withdrawal response" (Koob and Bloom 1988). Thus, a within-system neuroadaptation is a molecular or cellular

change within a given reward circuit to accommodate overactivity of hedonic processing associated with addiction resulting in a decrease in reward function.

The emotional dysregulation associated with the *withdrawal/negative affect* stage may also involve between-system neuroadaptations in which neurochemical systems other than those involved in the positive rewarding effects of drugs of abuse are recruited or dysregulated by chronic activation of the reward system. “In the between-systems opposing process, a different cellular system and separable molecular apparatus would be triggered by the changes in the primary drug response neurons and would produce the adaptation and tolerance” (Koob and Bloom 1988). Thus, a between-system neuroadaptation is a circuitry change in which another different circuit (anti-reward circuit) is activated by the reward circuit and has opposing actions, again limiting reward function. The remainder of this review explores the neuroadaptational changes that occur in the brain emotional systems to account for the neurocircuitry changes that produce opponent processes and are hypothesized to play a key role in the compulsivity of addiction.

2 Animal Models for Compulsive Alcohol Seeking

Methods of inducing binge-like drinking with alcohol range from having animals drink alcohol solutions that are made more palatable with the addition of a sweetener (Ji et al. 2008) to restricting intake to specific periods of the dark cycle (drinking in the dark; Rhodes et al. 2005) to models involving alcohol dependence in animals such as alcohol vapor inhalation, intragastric alcohol infusion, and alcohol-liquid diet. The compulsive use of alcohol derives from multiple sources of reinforcement, and animal models have been developed not only for the acute positive reinforcing effects of ethanol, but also for the negative reinforcing effects associated with removal of the aversive effects of ethanol withdrawal or an existing aversive state (i.e., self-medication of the aversive effects of abstinence from chronic ethanol or self-medication of a pre-existing negative affective state; Koob and Le Moal 1997). A major early breakthrough was the development of a training procedure involving access to a sweetened solution and a subsequent fading in of ethanol to avoid the aversiveness of the ethanol taste (for review, see Samson 1987). Subsequent work extended these procedures to measures of self-administration in dependent rats and post-dependent rats (Roberts et al. 1996; O’Dell et al. 2004).

High doses of alcohol solution will be self-administered intragastrically after animals are made dependent via passive intragastric infusion, and rats will self-infuse 4–7 g/kg per day of ethanol (Fidler et al. 2006). Here, blood alcohol levels average 0.12 g%, measured 30 min after the start of a bout in which rats infuse 1.5 g/kg per 30 min.

In an alcohol-liquid diet procedure, the diet is typically the sole source of calories available to rats (for example, see Moy et al. 1997), thereby forcing rats to consume the alcohol. Typically, rats are provided a palatable liquid diet containing 5–8.7% v/v ethanol as their sole source of calories sufficient to produce dependence and maintain

blood alcohol levels of 100–130 mg% during the dark (active drinking) cycle (Schulteis et al. 1996; Brown et al. 1998; Valdez et al. 2004). High responders during withdrawal from liquid diet will reach blood alcohol levels of approximately 80–100 mg% (Schulteis et al. 1996; Gilpin et al. 2009).

Reliable self-administration of ethanol in dependent animals using ethanol vapor exposure has been extensively characterized in rats, in which animals obtain blood alcohol levels in the 100–150 mg% range (Roberts et al. 1999, 2000). Similarly, rats with a history of alcohol dependence show increased self-administration of ethanol, even weeks after acute withdrawal (Roberts et al. 2000). In a variant of alcohol vapor exposure with more face validity, intermittent exposure to chronic ethanol using alcohol vapor chambers (14 h on/10 h off) produces more rapid escalation to increased ethanol intake and higher amounts of intake (O'Dell et al. 2004; Rimondini et al. 2002), and blood alcohol levels are reliably above 140 mg% after a 30 min session of self-administration in dependent animals (Richardson et al. 2008). In both the liquid diet and ethanol vapor procedures, alcohol intake is directly related to the blood alcohol range and the pattern of intermittent high-dose alcohol exposure (Gilpin et al. 2009). Although the alcohol vapor model may have limited face validity, considering that alcohol is passively administered to animals, numerous studies demonstrated that it also has robust predictive validity for alcohol addiction (Heilig and Koob 2007; Koob et al. 2009).

A similar procedure has been developed for mice and produces reliable increases in ethanol self-administration during withdrawal. Now termed chronic intermittent exposure (CIE), C57BL/6 mice are exposed to intermittent durations of ethanol vapor (three cycles of 16 h of vapor and 8 h of air) and then tested in a 2 h limited access ethanol preference drinking test during the circadian dark period (Becker and Lopez 2004; Lopez and Becker 2005; Finn et al. 2007). Intermittent ethanol vapor exposure significantly increased 15% (v/v) ethanol intake by 30–50% in the post-vapor period, usually after multiple cycles and usually after 24 h of withdrawal (Finn et al. 2007). Similar results have been reported using an operant response in mice in 60 min test sessions for 10% (w/v) ethanol with intermittent vapor exposure of 14 h on/10 h off (Chu et al. 2007).

3 Neural Substrates for the Negative Emotional State Associated with Alcoholism

3.1 *Within-System Neuroadaptations that Contribute to the Compulsivity Associated with the Dark Side of Alcoholism*

Within-system neuroadaptations to chronic drug exposure include decreases in function of the same neurotransmitter systems in the same neurocircuits implicated in the acute reinforcing effects of drugs of abuse. One prominent hypothesis is that dopamine systems are compromised in crucial phases of the addiction cycle, such

Table 1 Effects of intravenous self-administration of D-amphetamine, cocaine, and heroin and oral self-administration of alcohol on extracellular dopamine levels in the nucleus accumbens using in vivo microdialysis

Drug	% Increase in Dopamine over Baseline	Reference
D-Amphetamine	700%	Di Ciano et al (1995)
Cocaine	200–500%	Di Ciano et al (1995); Weiss et al (1992a)
Alcohol	25–50%	Weiss et al (1992b, 1996)
Heroin	<20%	Hemby et al (1995)

as withdrawal and protracted abstinence. This decrease in dopamine function is hypothesized to lead to decreased motivation for non-drug-related stimuli and increased sensitivity to the abused drug (Melis et al. 2005). Activation of the mesolimbic dopamine system has long been known to be critical for the acute rewarding properties of psychostimulant drugs and to be associated with the acute reinforcing effects of alcohol (Koob 1992; McBride and Li 1998; Nestler 2005). However, the magnitude of the increase in dopaminergic activity produced by alcohol pales in comparison to that of psychostimulant "intoxication." For example, intravenous cocaine self-administration produces a 200% increase in extracellular dopamine (Weiss et al. 1992b) compared with ethanol which produces a 20% increase in extracellular dopamine in the nucleus accumbens (Doyon et al. 2003) and heroin (which does not increase extracellular dopamine in the nucleus accumbens) (Table 1). Such a relationship changes with the development of dependence and may change with genetic background (see Ramachandani et al. 2010, who demonstrated a nearly 200% increase with alcohol in animals that carried the OPRM1 118G variant).

More compelling in the mesolimbic dopamine domain are the decreases in activity of the mesolimbic dopamine system and decreases in serotonergic neurotransmission in the nucleus accumbens that occur during alcohol withdrawal in animal studies (Rossetti et al. 1992; Weiss et al. 1992a, 1996). In dependent male Wistar rats trained to self-administer ethanol during withdrawal, the release of dopamine and serotonin was monitored by microdialysis in the nucleus accumbens at the end of a 3–5 week ethanol (8.7% w/v) liquid diet regimen, during 8 h of withdrawal, and during renewed availability of ethanol involving the opportunity to operantly self-administer ethanol (10% w/v) for 60 min, followed by unlimited access to the ethanol liquid diet. In nondependent rats, operant ethanol self-administration increased both dopamine and serotonin release in the nucleus accumbens. Withdrawal from the chronic ethanol diet produced a progressive suppression in the release of these transmitters over the 8 h withdrawal period. Self-administration of ethanol reinstated and maintained dopamine release at pre-withdrawal levels but failed to completely restore serotonin efflux. These findings suggested that deficits in nucleus accumbens monoamine release may contribute to the negative affective consequences of ethanol withdrawal and thereby motivate ethanol-seeking behavior in dependent subjects (Weiss et al. 1996). Similar

Table 2 Role of corticotropin-releasing factor in dependence

Drug	CRF antagonist effects on withdrawal-induced anxiety-like responses	Withdrawal-induced changes in extracellular CRF in CeA	CRF antagonist effects on dependence-induced increases in self-administration
Cocaine	↓	↑	↓
Opioids	↓[a]	↑	↓
Ethanol	↓	↑	↓
Nicotine	↓	↑	↓
Δ^9-Tetrahydrocannabinol	↓	↑	nt

[a] Aversive effects with place conditioning
nt, not tested
CeA, central nucleus of the amygdala

dramatic decreases in extracellular dopamine in the nucleus accumbens, measured by microdialysis, were found in a study in which animals were tested for 8 h into ethanol withdrawal produced by chronic repeated ethanol injections of up to 5 g/kg every 6 h for six consecutive days using the Majchrowicz procedure (Majchrowicz 1975; Rossetti et al. 1999). Thus, as a result, ethanol-dependent animals may show a much greater percentage increase in dopamine release in the nucleus accumbens during ethanol self-administration during withdrawal because baseline levels of dopamine are so low during withdrawal (Weiss et al. 1996).

Imaging studies in drug-addicted humans have consistently shown long-lasting decreases in the numbers of dopamine D_2 receptors in alcoholics compared with controls (Volkow et al. 2002). Additionally, alcohol-dependent subjects had dramatically reduced dopamine release in the striatum response to a pharmacological challenge with the stimulant drug methylphenidate (Volkow et al. 2007). Decreases in the number of dopamine D_2 receptors, coupled with the decrease in dopaminergic activity, in cocaine, nicotine, and alcohol abusers are hypothesized to produce a decreased sensitivity of reward circuits to stimulation by natural reinforcers (Martin-Solch et al. 2001; Volkow and Fowler 2000). These findings suggest an overall reduction in the sensitivity of the dopamine component of reward circuitry to natural reinforcers and other drugs in drug-addicted individuals (Table 2).

Other within-system neuroadaptations under this conceptual framework could include increased sensitivity of receptor transduction mechanisms in the nucleus accumbens. Drugs of abuse have acute receptor actions that are linked to intracellular signaling pathways that may undergo adaptations with chronic treatment. In the context of chronic alcohol administration, multiple molecular mechanisms have been hypothesized to counteract the acute effects of ethanol that could be considered within-system neuroadaptations. For example, chronic ethanol decreases γ-aminobutyric acid (GABA) receptor function, possibly through downregulation of the $\alpha 1$ subunit (Mhatre et al. 1993; Devaud et al. 1997). Chronic ethanol also decreases the acute inhibition of adenosine reuptake (i.e., tolerance develops to the inhibition of adenosine by ethanol; Sapru et al. 1994). Perhaps more relevant to the present treatise, whereas acute ethanol activates adenylate

cyclase, withdrawal from chronic ethanol decreases CREB phosphorylation in the amygdala and is linked to decrease in function of neuropeptide Y (NPY) and to the anxiety-like responses observed during acute ethanol withdrawal (Chance et al. 2000; Pandey 2004).

3.2 Between-System Neuroadaptations that Contribute to Compulsivity Associated with the Dark Side of Alcoholism

Brain neurochemical systems involved in arousal-stress modulation may also be engaged within the neurocircuitry of the brain stress systems in an attempt to overcome the chronic presence of the perturbing drug (alcohol) and to restore normal function despite the presence of drug. The neuroanatomical entity termed the extended amygdala (Heimer and Alheid 1991) may represent a common anatomical substrate integrating brain arousal-stress systems with hedonic processing systems to produce some of the between-system opponent process elaborated above. The extended amygdala is composed of the central nucleus of the amygdala, bed nucleus of the stria terminalis, and a transition zone in the medial (shell) subregion of the nucleus accumbens. Each of these regions has cytoarchitectural and circuitry similarities (Heimer and Alheid 1991). The extended amygdala receives numerous afferents from limbic structures, such as the basolateral amygdala and hippocampus, and sends efferents to the medial part of the ventral pallidum and a large projection to the lateral hypothalamus, thus further defining the specific brain areas that interface classical limbic (emotional) structures with the extrapyramidal motor system (Alheid et al. 1995). The extended amygdala has long been hypothesized to play a key role not only in fear conditioning (Le Doux 2000) but also in the emotional component of pain processing (Neugebauer et al. 2004).

The brain stress system mediated by corticotropin-releasing factor (CRF) systems in both the extended amygdala and hypothalamic–pituitary–adrenal axis are dysregulated by chronic administration of all major drugs with dependence or abuse potential, with a common response of elevated adrenocorticotropic hormone, corticosterone, and extended amygdala CRF during acute withdrawal from chronic drug administration (Rivier et al. 1984; Merlo-Pich et al. 1995; Koob et al 1994; Rasmussen et al. 2000; Olive et al. 2002; Delfs et al. 2000; Koob 2008a).

More specifically, alcohol withdrawal reliably produces anxiety-like responses in animal models that can be reversed by CRF receptor antagonists (Koob 2008a). Ethanol withdrawal produces anxiety-like behavior that is reversed by intracerebroventricular administration of CRF_1/CRF_2 peptidergic antagonists (Baldwin et al. 1991), small-molecule CRF_1 antagonists (Knapp et al. 2004; Overstreet et al. 2004; Funk et al. 2007), and intracerebral administration of a peptidergic CRF_1/CRF_2 antagonist into the amygdala (Rassnick et al. 1993). CRF antagonists injected intracerebroventricularly or systemically also block the potentiated anxiety-like responses to stressors observed during protracted abstinence from chronic ethanol (Breese et al. 2005; Valdez et al. 2003; Sommer et al. 2008).

Perhaps more relevant to the present thesis are studies showing that intermittent alcohol exposure sensitizes withdrawal of anxiety-like responses and that administration of drug treatments during withdrawal from the first and second alcohol cycles blocked this sensitization of withdrawal (Knapp et al. 2004). Diazepam, flumazenil (a $GABA_A$ receptor partial agonist), and baclofen (a $GABA_B$ receptor agonist) blocked the sensitization of withdrawal, consistent with a within-system neuroadaptation (Knapp et al. 2004, 2005, 2007; see above). However, a CRF_1 antagonist also prevented the sensitization of withdrawal-induced anxiety (Overstreet et al. 2004a, 2005). These results are consistent with a prolonged history of alcohol exposure producing persistent upregulation of both CRF and CRF_1 receptors in the brain (Roberto et al. 2010; Sommer et al. 2008; Zorrilla et al. 2001).

The ability of CRF antagonists to block the anxiogenic-like and aversive-like motivational effects of drug withdrawal would predict motivational effects of CRF antagonists in animal models of extended access to drugs. A particularly dramatic example of the motivational effects of CRF in dependence can be observed in animal models of ethanol self-administration in dependent animals. During ethanol withdrawal, extrahypothalamic CRF systems become hyperactive, with an increase in extracellular CRF within the central nucleus of the amygdala and bed nucleus of the stria terminalis in dependent rats (Funk et al. 2006; Merlo-Pich et al. 1995; Olive et al. 2002). The dysregulation of brain CRF systems is hypothesized to underlie not only the enhanced anxiety-like behaviors but also the enhanced ethanol self-administration associated with ethanol withdrawal. Supporting this hypothesis, the subtype nonselective CRF receptor antagonists α-helical $CRF_{9\text{-}41}$ and D-Phe $CRF_{12\text{-}41}$ (intracerebroventricular administration) reduced ethanol self-administration in dependent animals during acute withdrawal and during protracted abstinence (Valdez et al. 2002). When administered directly into the central nucleus of the amygdala, a CRF_1/CRF_2 antagonist blocked ethanol self-administration in ethanol-dependent rats (Funk et al. 2006). Systemic injections of small-molecule CRF_1 antagonists also blocked the increased ethanol intake associated with acute withdrawal and protracted abstinence (Gehlert et al. 2007; Funk et al. 2007). These data suggest an important role for CRF, primarily within the central nucleus of the amygdala, in mediating the increased self-administration associated with dependence. Consistent with the sensitization of the withdrawal response associated with repeated alcohol exposure, a CRF antagonist administered during repeated withdrawal also blocked the development of excessive drinking during withdrawal (Roberto et al. 2010).

Although less well developed, evidence supports a role of norepinephrine systems in the extended amygdala in the negative motivational state and increased self-administration associated with dependence. Substantial evidence has accumulated suggesting that in animals and humans, central noradrenergic systems are activated during acute withdrawal from ethanol. Alcohol withdrawal in humans is associated with activation of noradrenergic function, and the signs and symptoms of alcohol withdrawal in humans are blocked by postsynaptic β-adrenergic

blockade (Romach and Sellers 1991). Alcohol withdrawal signs are also blocked in animals by administration of α_1 antagonists and β-adrenergic antagonists and selective blockade of norepinephrine synthesis (Trzaskowska and Kostowski 1983). In dependent rats, the α_1 antagonist prazosin selectively blocked the increased drinking associated with acute withdrawal (Walker et al. 2008). Thus, converging data suggest that noradrenergic neurotransmission is enhanced during ethanol withdrawal and that noradrenergic functional antagonists can block aspects of ethanol withdrawal.

Dynorphin, an opioid peptide that binds to κ opioid receptors, has long been known to show activation with chronic administration of psychostimulants and opioids (Nestler 2004; Koob 2008a), and κ opioid receptor agonists produce aversive effects in animals and humans (Mucha and Herz 1985; Pfeiffer et al. 1986). Although κ agonists suppress nondependent drinking, possibly via aversive stimulus effects (Wee and Koob 2010), κ opioid antagonists block the excessive drinking associated with ethanol withdrawal and dependence (Holter et al. 2000; Walker and Koob 2008). Recently, some have argued that the effects of CRF in producing negative emotional states are mediated by activation of κ opioid systems (Land et al. 2008). However, κ receptor activation can activate CRF systems in the spinal cord (Song and Takemori 1992), and there is pharmacological evidence that dynorphin systems can also activate the CRF system. A CRF_1 antagonist blocked κ agonist-induced reinstatement of cocaine seeking in squirrel monkeys (Valdez et al. 2007).

The dynamic nature of the brain stress system response to challenge is illustrated by the pronounced interaction of central nervous system CRF systems and central nervous system norepinephrine systems. Conceptualized as a feed-forward system at multiple levels of the pons and basal forebrain, CRF activates norepinephrine, and norepinephrine in turn activates CRF (Koob 1999). Much pharmacologic, physiologic, and anatomic evidence supports an important role for a CRF-norepinephrine interaction in the region of the locus coeruleus in response to stressors (Valentino et al. 1991, 1993; Van Bockstaele et al. 1998). However, norepinephrine also stimulates CRF release in the paraventricular nucleus of the hypothalamus (Alonso et al. 1986), bed nucleus of the stria terminalis, and central nucleus of the amygdala. Such feed-forward systems were further hypothesized to have powerful functional significance for mobilizing an organism's response to environmental challenge, but such a mechanism may be particularly vulnerable to pathology (Koob 1999).

Neuropeptide Y is a neuropeptide with dramatic anxiolytic-like properties localized to the amygdala and has been hypothesized to have effects opposite to CRF in the negative motivational state of withdrawal from drugs of abuse (Heilig and Koob 2007). Significant evidence suggests that activation of NPY in the central nucleus of the amygdala can block the motivational aspects of dependence associated with chronic ethanol administration. Neuropeptide Y administered intracerebroventricularly blocked the increased drug intake associated with ethanol dependence (Thorsell et al. 2005a, b). Injection of NPY directly into the central nucleus of the amygdala (Gilpin et al. 2008) and viral vector-enhanced expression

of NPY in the central nucleus of the amygdala also blocked the increased drug intake associated with ethanol dependence (Thorsell et al. 2007).

Thus, acute withdrawal from drugs increases CRF in the central nucleus of the amygdala, which has motivational significance for the anxiety-like effects of acute withdrawal from alcohol and the increased drug intake associated with dependence. Acute withdrawal may also increase the release of norepinephrine in the bed nucleus of the stria terminalis and dynorphin in the nucleus accumbens, both of which may contribute to the negative emotional state associated with dependence. Decreased activity of NPY in the central nucleus of the amygdala may contribute to the anxiety-like state associated with ethanol dependence. Activation of brain stress systems (CRF, norepinephrine and dynorphin) combined with inactivation of brain anti-stress systems (NPY) elicits powerful emotional dysregulation in the extended amygdala. Such dysregulation of emotional processing may be a significant contribution to the between-system opponent processes that help maintain dependence and also set the stage for more prolonged state changes in emotionality such as in protracted abstinence.

4 Compulsivity in Alcoholism: an Allostatic View

Compulsivity in alcoholism can derive from multiple sources, including enhanced incentive salience, engagement of habit function, and impairment in executive function. However, underlying each of these sources is a negative emotional state that may strongly impact on compulsivity. The development of the negative emotional state that drives the negative reinforcement of addiction has been defined as the "dark side" of addiction (Koob and Le Moal 2005, 2008) and is hypothesized to be the *b-process* of the hedonic dynamic known as opponent process when the *a-process* is euphoria. The negative emotional state that comprises the *withdrawal/negative affect* stage consists of key motivational elements, such as chronic irritability, emotional pain, malaise, dysphoria, alexithymia, and loss of motivation for natural rewards, and is characterized in animals by increase in reward thresholds during withdrawal from all major drugs of abuse. Two processes are hypothesized to form the neurobiological basis for the *b-process*: loss of function in the reward systems (within-system neuroadaptation) and recruitment of the brain stress or anti-reward systems (between-system neuroadaptation; Koob and Bloom 1988; Koob and Le Moal 1997). Anti-reward is a construct based on the hypothesis that brain systems are in place to limit reward (Koob and Le Moal 2008). As dependence and withdrawal develop, brain stress systems, such as CRF, norepinephrine, and dynorphin, are recruited, producing aversive or stress-like states (Koob 2003; Nestler 2001; Aston-Jones et al. 1999). At the same time, within the motivational circuits of the ventral striatum-extended amygdala, reward function decreases. The combination of decreases in reward neurotransmitter function and recruitment of anti-reward systems provides a powerful source of

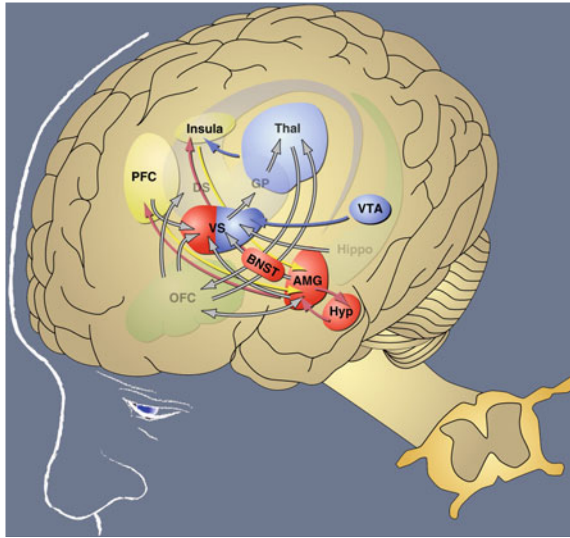

Fig. 3 Pathways for key elements of addiction circuitry implicated in negative emotional states. Addiction circuitry is composed of structures involved in the three stages of the addiction cycle: *binge/intoxication* (ventral striatum, dorsal striatum and thalamus), *withdrawal/negative affect* (ventral striatum, bed nucleus of the stria terminalis and central nucleus of the amygdala), *preoccupation/anticipation* (prefrontal cortex, orbitofrontal cortex and hippocampus). Highlighted here for the *withdrawal/negative affect* stage is increased activity in the extended amygdala and decreased activity in the reward system, illustrated with the use of imaging colors (i.e., *red* for high activity and *blue* for low activity). Modified with permission from Blackburn-Munro and Blackburn-Munro (2003) and Koob et al. (2008). AMG, amygdala; BNST, bed nucleus of the stria terminalis; DS, dorsal striatum; GP, globus pallidus; Hippo, hippocampus; Hyp, hypothalamus; Insula, insular cortex; OFC, orbitofrontal cortex; PFC, prefrontal cortex; Thal, thalamus; VS, ventral striatum; and VTA, ventral tegmental area. [Modified with permission from Zald and Kim 2001]

negative reinforcement that contributes to compulsive drug-seeking behavior and addiction (Fig. 3).

An overall conceptual theme argued here is that drug addiction represents a break with homeostatic brain regulatory mechanisms that regulate the emotional state of the animal. The dysregulation of emotion begins with the binge and subsequent acute withdrawal, but leaves a residual neuroadaptive trace that allows rapid "re-addiction" even months and years after detoxification and abstinence. Thus, the emotional dysregulation of alcohol addiction represents more than simply a homeostatic dysregulation of hedonic function—it also represents a dynamic break with homeostasis of this system that has been termed *allostasis* (Koob 2003).

Allostasis, originally conceptualized to explain persistent morbidity of arousal and autonomic function, can be defined simply as "stability through change" (Sterling and Eyer 1988). Allostasis is different from homeostasis. Allostasis

involves a feed-forward mechanism rather than the negative feedback mechanisms of homeostasis. Allostasis involves a changed set point with continuous re-evaluation of need and continuous readjustment of all parameters toward new set points. The set point in question here is emotional state. An *allostatic state* can be defined as a state of chronic deviation of the reward system from its normal (homeostatic) operating level. *Allostatic load* has been defined as the "long-term cost of allostasis that accumulates over time and reflects the accumulation of damage that can lead to pathological states" (McEwen 2000). Although the concept of allostatic state has not received much attention, the argument here is that alcoholism reflects largely a movement to an allostatic state, often before sufficient pathology has ensued to produced allostatic load sufficient for physical pathology (Koob and Le Moal 2001).

Allostatic mechanisms have been hypothesized to be involved in maintaining a functioning brain reward system that has relevance for the pathology of addiction (Koob and Le Moal 2001). Two components are hypothesized to adjust to challenges of the brain produced by drugs of abuse: underactivation of brain reward transmitters and circuits and recruitment of the brain anti-reward or brain stress systems (Fig. 4). Thus, the very physiological mechanism that allows rapid responses to environmental challenge becomes the source of pathology if adequate time or resources are not available to shut off the response (one example is the interaction between CRF and norepinephrine in the brainstem and basal forebrain that could lead to pathological anxiety; Koob 1999).

Repeated challenges, such as with repeated alcohol binges, lead to attempts of the brain via molecular, cellular, and neurocircuitry changes to maintain stability but at a cost. For the alcoholism framework elaborated here, the residual deviation from a normal emotional state is termed the *allostatic state*. This state represents a combination of chronic elevation of the reward set point fueled by decreased function of reward circuits and recruitment of anti-reward systems, both of which lead to the compulsivity of alcohol-seeking and alcohol taking. How these systems are modulated by other known brain emotional systems localized to the basal forebrain, where the ventral striatum and extended amygdala project to convey emotional valence, how the dysregulation of brain emotional systems impacts on the cognitive domain linked to impairments in executive function, and how individuals differ at the molecular-genetic level of analysis to convey loading on these circuits remain challenges for future research (George and Koob 2010).

As such, the present thesis does not preclude a key role for other systems associated with the addiction process, including the mesolimbic dopamine system involved in incentive salience, the dorsal striatum involved in habit formation, the parabrachial amygdala and spinothalamocortical systems involved in pain, and the prefrontal cortex involved in decision-making (Koob and Volkow 2010; George and Koob 2010). Such modules are driven by bottom-up signals from both the external world and interoceptive signals and by top-down signals from higher-order systems mediating cognitive control. Indeed, the failure of a specific module may differ from one individual to another and may represent a neuropsychobiological mechanism underlying individual differences in the vulnerability to drug

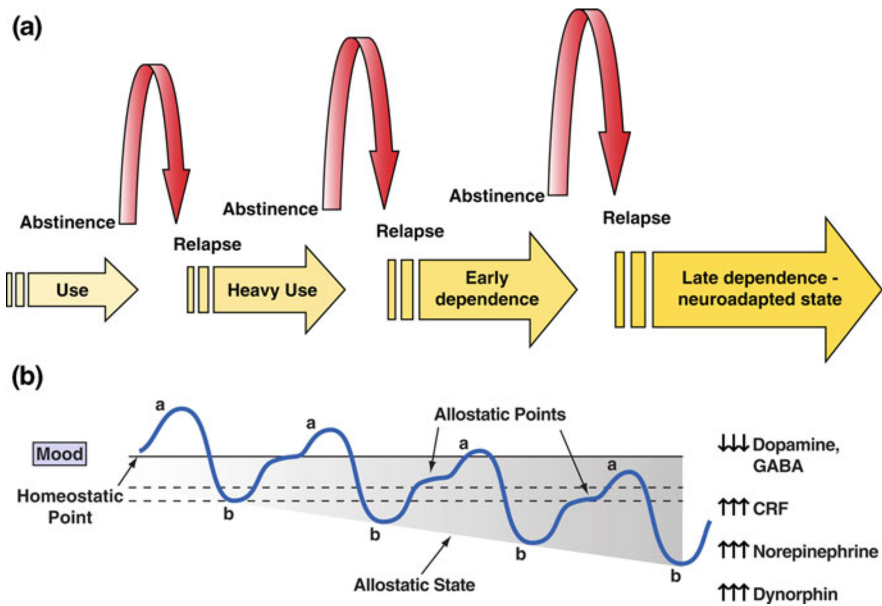

Fig. 4 **a** Schematic of the progression of alcohol dependence over time, illustrating the shift in underlying motivational mechanisms. From initial, positive reinforcing, pleasurable alcohol effects, the addictive process progresses over time to being maintained by negative reinforcing relief from a negative emotional state. Data presented in this paper suggest that neuroadaptations encompassing the recruitment of extrahypothalamic CRF systems are key to this shift. (Taken with permission from Heilig and Koob 2007.) **b** The *a-process* represents a positive hedonic or positive mood state, and the *b-process* represents the negative hedonic or negative mood state. The affective stimulus (state) has been argued to be the sum of both the *a-process* and *b-process*. An individual who experiences a positive hedonic mood state from a drug of abuse with sufficient time between re-administering the drug is hypothesized to retain the *a-process*. An appropriate counteradaptive opponent process (*b-process*) that balances the activational process (*a-process*) does not lead to an allostatic state. The changes in the affective stimulus (state) in an individual with repeated frequent drug use may represent a transition to an allostatic state in the brain systems and, by extrapolation, a transition to addiction (*see* text). Notice that the apparent *b-process* never returns to the original homeostatic level before drug taking begins again, thus creating a greater and greater allostatic state in the brain emotional systems. The counteradaptive opponent-process (*b-process*) does not balance the activational process (*a-process*) but in fact shows a residual hysteresis. Although these changes illustrated in the figure are exaggerated and condensed over time, the hypothesis is that even during post-detoxification (a period of "protracted abstinence"), the brain emotional systems still bear allostatic changes (*see* text). The following definitions apply: *allostasis*, the process of achieving stability through change; *allostatic state*, a state of chronic deviation of the regulatory system from its normal (homeostatic) operating level; *allostatic load*, the cost to the brain and body of the deviation, accumulating over time, and reflecting in many cases pathological states and accumulation of damage. [Modified with permission from Koob and Le Moal 2001.]

addiction. For example, we have hypothesized that individual differences in the function of the incentive salience mesolimbic dopamine system and the habit/ striatum modules may be particularly important for craving-type 1 (or reward

craving), defined as craving for the rewarding effects of alcohol and usually induced by stimuli that have been paired with alcohol self-administration, such as environmental cues. Additionally, hypoactivity of the decision-making/prefrontal cortex module may lead to a loss of control over drug intake despite negative consequence because of impaired inhibitory control and decision-making leading to choices of immediate rewards over delayed rewards (Goldstein and Volkow 2002).

Nevertheless the hypothesis outlined here is that a core component of alcoholism involves hyperactivity of the negative emotional state/extended amygdala system that is associated with increased emotional pain and stress and might be a risk factor for drug use as self-medication for emotional pain, dysphoria, and stress (Khantzian 1997). A subhypothesis is that vulnerability in the emotional pain parabrachial-amygdala system (Besson 1999; Shurman et al. 2010) may lead to increased emotional pain during withdrawal and intense craving-type 2 (or withdrawal relief craving), which is conceptualized as an excessive motivation for the drug to obtain relief from a state change characterized by anxiety and dysphoria after protracted abstinence (Heinz et al. 2003), thus contributing to the preponderant role of the *withdrawal/negative affect* stage that characterizes alcoholism. Increased reactivity of the stress/hypothalamic–pituitary–adrenal axis module may be critical in the initiation of alcohol intake and for the maintenance of drug intake which have little initial rewarding value, such as nicotine. Activation of the hypothalamic–pituitary–adrenal axis can potentiate the reinforcing effects of drugs (Piazza and Le Moal 1998). However, this activation can in turn drive amygdala CRF, further exacerbating the development of negative emotional states (Koob and Kreek 2007). Although the initial deficit in a specific functional circuit that drives excessive drinking might be specific to one stage of the addiction cycle, as the transition to addiction progresses, an individual is ultimately likely to show a progressive and generalized loss of control over many, if not all, systems. However, the thesis argued here is that as excessive alcohol intake progresses to Substance Dependence on Alcohol (Alcoholism), a common dysregulated functional element is a reward system deficit.

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Synaptic Effects Induced by Alcohol

David M. Lovinger and Marisa Roberto

Abstract Ethanol (EtOH) has effects on numerous cellular molecular targets, and alterations in synaptic function are prominent among these effects. Acute exposure to EtOH activates or inhibits the function of proteins involved in synaptic transmission, while chronic exposure often produces opposing and/or compensatory/homeostatic effects on the expression, localization, and function of these proteins. Interactions between different neurotransmitters (e.g., neuropeptide effects on release of small molecule transmitters) can also influence both acute and chronic EtOH actions. Studies in intact animals indicate that the proteins affected by EtOH also play roles in the neural actions of the drug, including acute intoxication, tolerance, dependence, and the seeking and drinking of EtOH. This chapter reviews the literature describing these acute and chronic synaptic effects of EtOH and their relevance for synaptic transmission, plasticity, and behavior.

Keywords GABA · Glutamate · Monoamine · Neuropeptide · Neurotransmitter receptor · Presynaptic · Postsynaptic · Protein phosphorylation · Synaptic plasticity · Intoxication · Tolerance · Dependence

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D. M. Lovinger (✉)
Laboratory for Integrative Neuroscience, NIAAA, 5625 Fishers Lane,
Room TS-13A, Rockville, MD 20852, USA
e-mail: lovindav@mail.nih.gov

M. Roberto
Committee on Neurobiology of Addictive Disorders, The Scripps Research Institute,
10550 N Torrey Pines Rd., SP30-1160, La Jolla, CA 92037, USA
e-mail: mroberto@scripps.edu

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Contents

1 Acute Ethanol Actions 32
1.1 Ligand-Gated Ion Channels and Postsynaptic Ethanol Effects 32
1.2 G protein-Coupled Receptors and Roles in Ethanol Effects 37
1.3 Presynaptic Effects of Ethanol 39
1.4 Monoamines and Neurotransmitter Transport 40
1.5 Ethanol and Synaptic Plasticity 41
2 Chronic Ethanol Actions 42
2.1 Chronic Ethanol Effects on Glutamatergic Transmission and Glutamate Roles in Synaptic Plasticity 42
2.2 Chronic Ethanol and GABAergic Transmission: Postsynaptic Effects 45
2.3 Chronic Ethanol and GABAergic Transmission: Presynaptic Effects 52
2.4 γ-Aminobutyric Acid$_B$ Receptors and Chronic Ethanol Actions 54
3 Neuropeptide Roles in Acute and Chronic Alcohol Actions 56
3.1 Corticotropin-Releasing Factor 56
4 Conclusions 66
References 67

1 Acute Ethanol Actions

Ethanol (EtOH) produces intoxication through actions on the central nervous system (CNS) at concentrations ranging from low to ~100 mM (at least in non-tolerant humans and experimental animals). A number of proteins involved in synaptic transmission are altered by EtOH effects within this concentration range. The target proteins include, but are not limited to, ion channels, neurotransmitter receptors, and intracellular signaling proteins. The first section of this article will review the literature describing the most prominent acute EtOH effects on synaptic transmission in the CNS. This review is not meant to be comprehensive, but rather to cover those effects that have been observed most consistently and are thought to contribute to intoxication.

1.1 Ligand-Gated Ion Channels and Postsynaptic Ethanol Effects

Ion channels are among the best characterized targets for acute EtOH actions (Lovinger 1997; Vengeliene et al. 2008). Ligand-gated ion channels (LGICs) are heteromeric proteins that bind extracellular neurotransmitters or intracellular messengers and transduce that binding energy into opening of an intrinsic ion pore (Collingridge et al. 2009). Among those channels activated by extracellular neurotransmitters there are three classes.

1.1.1 Cys-loop Ligand-Gated Ion Channels

The "cys-loop" LGICs are pentameric proteins characterized by an obligatory cysteine double bond in the N-terminal binding domain. Each subunit protein contains an extracellular ligand-binding domain, four membrane spanning domains, and one large intracellular loop domain that also serves as a "portal" for ion permeability. This receptor class includes proteins with cation-permeable pores, the nicotinic acetylcholine (nAChR) and serotonin$_3$ (5-HT$_3$) receptors, as well as those with anion-permeable pores, the γ-aminobutyric acid$_A$ (GABA$_A$), and strychnine-sensitive glycine (GlyR) receptors. This class of receptors is distributed throughout the peripheral and central nervous systems.

Generally, acute EtOH exposure enhances the function of cys-loop LGICs (Aguayo et al. 2002; Harris 1999; Lovinger 1997; Perkins et al. 2010), but instances of inhibition of the nAChRs and GABA$_A$Rs have been reported (Aguayo et al. 2002; Cardoso et al. 1999; Davis and De Fiebre 2006; Marszalec et al. 1994; Roberto et al. 2003). The most common EtOH action is to potentiate channel opening in the presence of a low concentration of agonist by increasing the probability of channel opening (Zhou et al. 1998), and/or increasing agonist affinity (Tonner and Miller 1995; Welsh et al. 2009). This potentiating effect can influence both synaptic and extrasynaptic receptors (Sebe et al. 2003; Ye et al. 2001; Eggers and Berger 2004; Ziskind-Conhaim et al. 2003) (Fig. 1). For example, EtOH has been shown to increase the amplitude and/or duration of GABA$_A$ and GlyR-mediated inhibitory postsynaptic currents (IPSCs) (Sebe et al. 2003; Ziskind-Conhaim et al. 2003).

EtOH potentiation of GABA$_A$ receptor function has been extensively studied. There are 19 subunit proteins that contribute to the formation of GABA$_A$ receptors (International Union of Basic and Clinical Pharmacology, IUPHAR, database http://www.iuphar-db.org/index.jsp). Many of these subunit combinations have been examined for function and pharmacology in heterologous expression systems. To briefly summarize a large body of data, there is evidence that EtOH potentiates the function of $\alpha/\beta/\gamma$-subunit-containing receptors, as well as those containing $\alpha4$ or $\alpha6$ along with β and δ subunits (Olsen et al. 2007; Lobo and Harris 2008; Mihic and Harris 1995; McCool et al. 2003). However, none of these findings has been uniformly replicated in all laboratories that have examined EtOH effects in heterologous systems (reviewed in Lovinger and Homanics 2007; Aguayo et al. 2002). Using cultured and isolated neurons, several investigators have observed potentiation of GABA$_A$R function (Celentano et al. 1988; Reynolds and Prasad 1991; Aguayo 1990; Nishio and Narahashi 1990; Sapp and Yeh 1998), but this sort of effect has not been observed in every neuronal type examined (e.g. McCool et al. 2003; White et al. 1990; Yamashita et al. 2006). A tonic GABA$_A$-mediated current is observed in many CNS neurons, and is thought to reflect the function of extrasynaptic, high affinity GABA receptors containing the δ receptor subunit (Hanchar et al. 2005). The Potentiation of this tonic current has been observed in recordings from cerebellum, hippocampus, and thalamus using

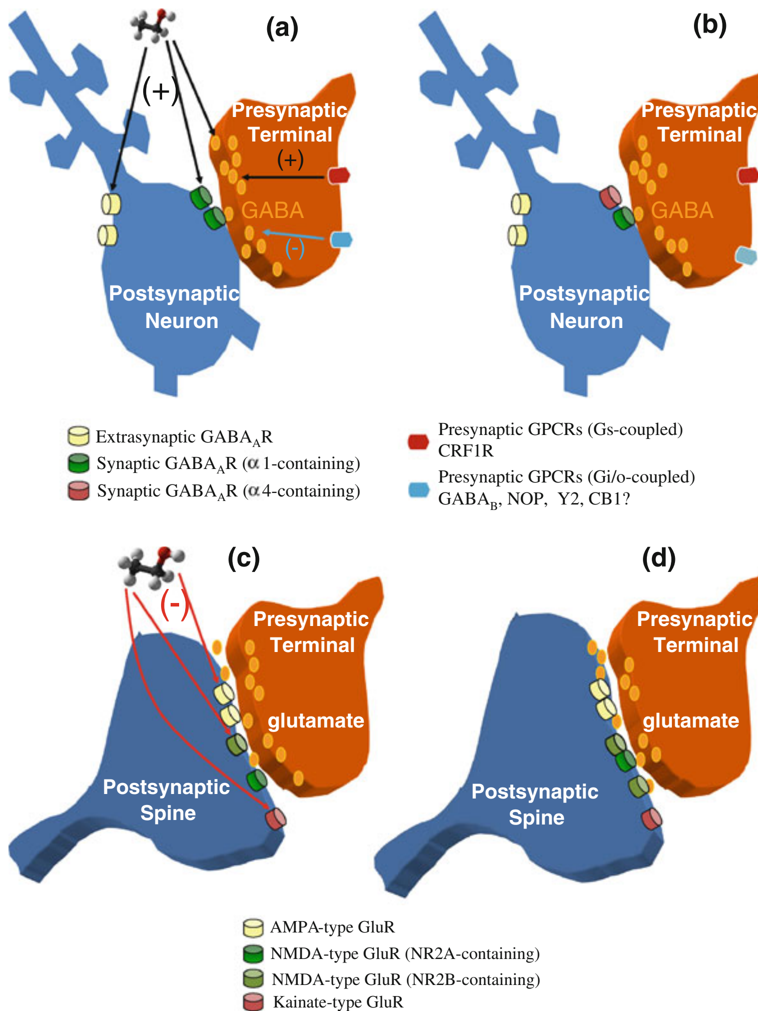

Fig. 1 Acute and chronic EtOH effects on GABAergic and glutamatergic synaptic transmission. **a** Schematic diagram of a GABAergic synapse, including presynaptic GPCRs that modulate neurotransmitter release, and postsynaptic ionotropic receptors (located both at synapses and extrasynaptically) that mediate fast synaptic transmission. The predominant presynaptic effect of acute EtOH is potentiation of GABA release (most likely by increasing the probability of vesicle fusion). This presynaptic potentiation may involve neuromodulators such as CRF, and activation of presynaptic GPCRs and downstream signaling pathways. Postsynaptically, EtOH potentiates ionotropic GABA$_A$ receptor function. Increases in synaptic GABA$_A$R function prolong synaptic responses, while potentiation of extrasynaptic receptors increases tonic current that affects neuronal excitability, **b** Changes in GABAergic synapses following chronic EtOH exposure. Presynaptically, the release of GABA is decreased. Alterations in levels of neuromodulators that act on GPCRs, as well as altered function of presynaptic GPCRs may contribute to these changes. Postsynaptically, the subunit composition of GABA$_A$Rs is altered, often including increased synaptic α4-containing receptors, and fewer α1-containing synaptic receptors. Synaptic

◀ **Fig. 1** (continued)
α4-containing receptors may be less sensitive to acute EtOH, promoting tolerance to synaptic effect of the drug, **c** Schematic diagram of a glutamatergic synapse on a dendritic spine, including postsynaptic ionotropic receptors that mediate fast synaptic transmission. The predominant effect of acute EtOH is to inhibit ionotropic glutamate receptor function, and all subclasses of these receptors are sensitive to EtOH inhibition. The most potent effects have been observed at kainate and NMDA receptor subtypes, **d** Changes in glutamatergic synapses following chronic EtOH exposure. Presynaptically, the release of glutamate is enhanced. Postsynaptically, NMDAR function is increased, most likely due to increased receptor density at the synapse. There is also evidence for increased numbers of NR2B-containing NMDARs. There is also evidence of increased volume of the dendritic spine

the brain slice preparation (Hanchar et al. 2005; Wei et al. 2004; Glykys et al. 2007; Jia et al. 2008, although see Botta et al. 2007).

Recent studies suggest that EtOH potentiation of $GABA_A$ receptor function depends on protein phosphorylation. Messing and co-workers have shown that activity of the epsilon subunit of protein kinase C (PKC) is necessary for EtOH potentiation of γ2-subunit-containing $GABA_A$ receptors expressed heterologously in a mammalian cell line (Qi et al. 2007). This PKC action appears to involve phosphorylation of a specific serine residue on the γ2 subunit. This finding may explain data from previous studies indicating the involvement of PKC in EtOH potentiation of GABAergic transmission (Weiner et al. 1994). However, in this earlier study it was not clear if the EtOH effects on transmission involved pre or postsynaptic mechanisms. A parallel line of investigation indicates that PKCδ is necessary for EtOH potentiation of tonic current involving δ-subunit-containing $GABA_A$Rs (Choi et al. 2008). It is not yet clear whether acute EtOH exposure activates PKC phosphorylation of the $GABA_A$R or whether phosphorylation on key amino acid residues is permissive for EtOH potentiation of receptor function, and this will be an interesting topic for future research.

EtOH potentiation of glycine-activated chloride channels appears to be dependent on receptor subunit composition. Potentiation is consistently greater at receptors containing the α1 subunit (Davies et al. 2003; Mascia et al. 1996; Mihic et al. 1997), at least when expressed in xenopus laevis oocytes (Valenzuela et al. 1998b, although see McCool et al. 2003; Yevenes et al. 2008). Receptors containing the α2 subunit also exhibit EtOH potentiation (McCool et al. 2003), but may be less sensitive than those containing the α1 subunit (Mascia et al. 1996). Inclusion of the β subunit along with α2 eliminates potentiation (McCool et al. 2003). Potentiation has also been observed in neurons from the brain and spinal cord, particularly in regions where the α1 subunit is expressed (Aguayo et al. 1996; Ye et al. 2001). Potentiation of the function of $GABA_A$ and glycine receptors is thought to increase inhibition of neurons. The relative influence of effects on synaptic versus extrasynaptic channels in producing this inhibition remains to be determined.

Acute EtOH exposure potentiates the function of 5-HT_3 receptors that contain an intrinsic cation channel (Lovinger 1991; Machu and Harris 1994). It is yet to be determined whether this action alters pre or postsynaptic mechanisms activated by this receptor.

1.1.2 Ionotropic Glutamate Receptors

The ionotropic glutamate receptors (iGluRs) constitute the second class of neurotransmitter-activated LGICs. Three major classes of iGluRs exist, the AMPA receptors (AMPARs, gene name GRIA, made by GluRs1-4), the NMDA receptors (NMDARs1-3, gene name GRIN), and the kainate receptors (KARs, made by GluRs5-7 and KAs1-2, gene name GRIK). These receptors are now thought to be tetrameric and each subunit contains a large N-terminal domain and an extracellular loop domain that together participate in ligand binding via a “venus fly-trap” motif (Gouaux 2004). The subunits have three membrane-spanning domains and a re-entrant pore-loop that forms the ion conduction pathway, as well as intracellular loops and a large intracellular C-terminal domain. The iGluRs are all cation-permeable, with varying ratios of Na^{+}, K^{+} and Ca^{2+} selectivity. These receptors are present on all CNS neurons, where they mediate fast synaptic transmission and activation of intracellular signaling.

EtOH has consistent inhibitory actions on iGluRs (although see Lu and Yeh 1999) (Fig. 1c, d). Inhibition of NMDARs at EtOH concentrations associated with intoxication is the best characterized of these effects (Criswell et al. 2003; Dildy and Leslie 1989; Hoffman et al. 1989; Lima-Landman and Albuquerque 1989; Lovinger et al. 1989). The synaptic responses mediated by NMDARs are also reduced by EtOH (Lovinger et al. 1990; Morrisett and Swartzwelder 1993; Roberto et al. 2004b; Wang et al. 2007).

Functional NMDARs always contain an obligatory NR1 subunit in combination with at least one NR2 or NR3 subunit. While EtOH inhibits all NMDAR subtypes, differences in the sensitivity to inhibition have been observed for recombinant with receptors containing different subunit compositions. The most common observation is that EtOH is less potent at receptors containing the NR1/2C composition in comparison to those containing NR1/2A or NR1/2B (Masood et al. 1994; Chu et al. 1995, but see Kuner et al. 1993; Lovinger 1995). There are several splice variants of the NR1-subunit, and a recent comprehensive study by Woodward and co-workers showed that the NR1 splicing status, in combination with the identity of the co-assembled NR2 subunit, has small but reliable effects on EtOH sensitivity (Jin and Woodward 2006). This NR1 splice variant effect could account for the previous difference in reports of low EtOH sensitivity of NR2C-containing receptors. Receptors containing the NR3 subunit are relatively insensitive to inhibition by EtOH, but inclusion of the NR2B-subunit enhances the EtOH inhibitory action on NR3-containing receptors (Jin et al. 2008). In addition, Mg^{2+} enhances EtOH inhibition of several NR1/2 and N1/2/3 receptor combinations, especially when NR2B is present (Jin et al. 2008). This finding may account for the larger effect of EtOH on NR2B containing NMDARs seen in some neuronal preparations (e.g. Fink and Göthert 1996; Lovinger 1995).

EtOH also inhibits the function of AMPARs, and effects can be seen at concentrations as low as 10 mM (Akinshola 2001; Akinshola et al. 2003; Dildy-Mayfield and Harris 1992; Möykkynen et al. 2003; Nieber et al. 1998; Wirkner et al. 2000). In neurons from the brain, EtOH generally shows lower

potency for inhibition of AMPARs in comparison to NMDARs (Frye and Fincher, 2000; Lovinger et al. 1989; Lovinger 1995). The EtOH sensitivity of recombinant AMPAR receptors is not greatly altered by changing the receptor subunit composition (Lovinger 1993), although the potency of EtOH is slightly higher for inhibition of GluR1-containing in contrast to GluR3-containing GluRs in *X. laevis* oocytes (Akinshola 2001). In addition, recombinant AMPA receptors containing GluRs 2 and 3 exhibit slightly decreased EtOH sensitivity in comparison to those containing GluRs1, 2, and 3 or 3 alone (Akinshola et al. 2003). Recent studies suggest that this EtOH action involves increased receptor desensitization (Möykkynen et al. 2003, 2009), and thus the drug has little impact on AMPAR-mediated synaptic responses at most synapses given that desensitization does not contribute to the amplitude or time course of excitatory postsynaptic currents (EPSCs) (Lovinger et al. 1990; Ariwodola et al. 2003, but see Nie et al. 1993, 1994; Roberto et al. 2004b; Mameli et al. 2005; Zhu et al. 2007).

Inhibition of KAR-mediated responses has been observed at quite low EtOH concentrations (Costa et al. 2000; Lack et al. 2008; Valenzuela et al. 1998a; Weiner et al. 1999). However, direct examination of KAR-mediated ion current has yielded mixed results, at least for the receptor constructs examined to date (Dildy-Mayfield and Harris 1992; Valenzuela et al. 1998a). Thus, it is not yet clear whether EtOH inhibition of KAR function involves a direct effect on protein function or a more indirect action. EtOH inhibition of iGluRs is generally thought to dampen neuronal excitability in many brain regions by reducing excitatory synaptic drive and inhibiting synaptic plasticity that requires iGluR activation.

1.1.3 Purinergic Ligand-Gated Ion Channels

The third major subtype of LGIC is the P2X purinergic receptor subclass. P2X receptors are trimeric (Mio et al. 2005) with each subunit containing an N-terminal ligand binding domain, two membrane-spanning domains linked by an extracellular ligand binding domain, and a C-terminal intracellular domain of moderate length. The second membrane-spanning domain appears to serve as the lining for the ion conduction pathway. EtOH inhibits the function of most P2X receptor subtypes, with some effects reported at concentrations associated with intoxication (Davies et al. 2002; Li et al. 1993). The P2X4 receptor appears to be the most sensitive to inhibition by EtOH, while P2X3 receptors exhibit EtOH-induced potentiation (Davies et al. 2002, 2005). At present, the physiologic consequences of P2X inhibition are unclear.

1.2 G protein-Coupled Receptors and Roles in Ethanol Effects

The majority of neurotransmitter receptors are members of the G protein-coupled receptor (GPCR) superfamily. These receptors are specialized for binding a neurotransmitter, and this binding stimulates rearrangement of the protein to favor

activation of intracellular signaling proteins known to bind GTP and GDP. In the GTP-bound state, the G protein is activated. Several forms of intracellular signaling proteins are affected by activated G proteins, including proteins that generate small molecule second messengers, as well as protein kinases and ion channels. Thus, G protein activation can affect neurophysiology fairly directly by altering ion channel function, and can have a long-lasting influence on neuronal function by altering intracellular signaling and even gene expression.

Receptor-activated G proteins are heterotrimeric, consisting of α, β, and γ subunits. The β and γ subunits form a tight complex, but when the G protein is activated the α subunit affinity for the β/γ complex is reduced. The result is that two signaling elements arise from the G protein activation and can act on different intracellular targets. The GPCRs act predominantly on three G protein subclasses: Gi/o, Gq-like, and Gs-like (Wickman and Clapham 1995). The Gi/o G protein class has net inhibitory effects on neuronal function, through actions of both the α and β/γ protein subunits. For example, the α subunit inhibits the enzyme adenylyl cylase (AC) that normally generates the second messenger cAMP. The β/γ subunits activate potassium channels that inhibit neuronal activity (the so-called G protein-activated inward rectifier, GIRK, potassium channels). The β/γ subunits also inhibit the function of voltage-gated calcium channels, leading to inhibition of neurotransmitter release, and also appear to have more direct effects on vesicle fusion (Dolphin 2003; Elmslie 2003; Miller 1998; Wu and Saggau 1994). The Gq-like α subunits activate protein and lipid signaling pathways that activate ion channels that excite neurons, inhibit potassium channels, and increase neurotransmitter release. Thus, activation of the Gq-subclass generally has a net excitatory effect on neuronal activity and synaptic transmission. The proximal effects of Gs-like G protein activation are not always clear. The α subunit of these G proteins stimulates AC/cAMP formation which can enhance synaptic transmission and inhibits some potassium channels. The effects on ion channel function of the different G proteins are outlined in detail in previous review articles (Dolphin 2003; Elmslie 2003; Wickman and Clapham 1995).

Direct effects of acute EtOH on the function of GPCRs and G proteins are generally weak. Furthermore, the physiologic impact of these actions is not always clear. However, there are mechanisms involving these molecules that are influenced by EtOH. Studies beginning in the 1980s showed that EtOH can stimulate cAMP formation (Luthin and Tabakoff 1984; Rabin and Molinoff 1981). This may be due to direct EtOH actions on AC, but other proteins that influence GPCRs and their signaling might play roles in the neural actions of EtOH (Bjork et al. 2008). The physiologic consequences of this AC activation have long been unclear. However, recent studies indicate that acute EtOH exposure can increase neurotransmitter release (described in greater detail later in this review, Fig. 1), and activation of AC is a strong candidate to mediate these effects (Kelm et al. 2008).

In heterologous expression systems, EtOH has been shown to inhibit responses to activation of GPCRs that couple to Gq-like G proteins. These findings mostly involve demonstrations that pharmacologically relevant concentrations of EtOH reduce the ability of the GPCRs to activate a calcium-dependent chloride current

in the *Xenopus laevis* oocyte preparation (Minami et al. 1997a, b, 1998). Among the GPCRs that have been examined in this context are metabotropic glutamate receptors (mGluRs), muscarinic ACh receptors and serotonin type 2 receptors. The observation that these three receptor effects are all inhibited despite differences in the structures of the receptor molecules themselves, indicates that the EtOH target site is likely downstream of the receptor itself. Indeed there is some evidence for involvement of protein kinase C, at least in the inhibition of muscarinic AChR-induced responses (Minami et al. 1997b).

EtOH can also potentiate the function of GIRK-type potassium channels (Aryal et al. 2009; Kobayashi et al. 1999; Lewohl et al. 1999). This effect occurs at concentrations associated with intoxication. The net effect of GIRK activation is to inhibit neuronal activity. This action of EtOH was originally observed in cerebellar granule neurons (Lewohl et al. 1999), and subsequent studies have indicated similar actions in midbrain dopaminergic neurons (Federici et al. 2009). EtOH effects on this G protein target may contribute to intoxication. Studies by Blednov et al. (2001) indicate that loss of the GIRK2 channel subunit alters acute EtOH actions. There is certainly a need for additional studies of how GIRK activation might contribute to intoxication.

1.3 Presynaptic Effects of Ethanol

EtOH potentiation of GABAergic synaptic inhibition is now known to result from both pre and postsynaptic actions. As discussed in the section on LGICs, the postsynaptic effects result from potentiation of $GABA_A$/anion channels. Recent studies indicate that EtOH also acts to enhance GABA release from presynaptic terminals, and this action contributes to enhanced synaptic inhibition (reviewed in Siggins et al. 2005) (Fig. 1). Increases in fast GABAergic synaptic transmission during EtOH treatment have been observed in cerebellum, hippocampus, ventral tegmental area (VTA), hypoglossal nucleus, and amygdala, both basolateral and central nuclei (Ariwodola and Weiner 2004; Ming et al. 2006; Kelm et al. 2007; Theile et al. 2008; Zhu and Lovinger 2006; Roberto et al. 2003; Sebe et al. 2003; Ziskind-Conhaim et al. 2003). These studies have been carried out mostly in brain slices and isolated brain neurons. Examination of spontaneous and miniature GABAergic IPSCs allows investigators to determine whether the frequency of synaptic events is altered (a likely presynaptic change), or whether the amplitude is affected (likely a postsynaptic change). Such analyses have consistently shown that sISPC and mIPSC frequencies are increased at EtOH concentrations associated with intoxication, at least in the amygdala, cerebellum, hippocampus and VTA (Ariwodola and Weiner 2004; Zhu and Lovinger 2006; Theile et al. 2008; Roberto et al. 2003; Kelm et al. 2007). These effects are rapid at onset and rapidly reversible following EtOH removal from tissue.

At present, little is known about the mechanisms underlying EtOH potentiation of GABA release. The increase in mIPSC frequency suggests that the site of EtOH

action is downstream of action potential generation and calcium entry into the presynaptic terminal. Experiments in the cerebellum and VTA suggest that EtOH interacts with mechanisms involved in intracellular calcium release, perhaps increasing calcium concentrations in the presynaptic terminal (Kelm et al. 2007; Theile et al. 2009). It would be helpful to know whether EtOH increases calcium concentrations in the relevant population of GABAergic presynaptic terminals. However, this is difficult to determine given the small size (<1 μM diameter) of terminals, and the diversity of subtypes of terminals found on any given neuron.

The role of intracellular signaling pathways in this potentiating EtOH effect has also been examined. It is well established that activation of AC or PKC potentiates transmission at synapses throughout the nervous system (see Leenders and Sheng 2005; Nguyen and Woo 2003 for review). Thus, it is logical to speculate that these signaling molecules might play a role in the acute alcohol action. Potentiation of GABA release onto cerebellar Purkinje neurons is eliminated in the presence of AC and protein kinase A (PKA) inhibitors (Kelm et al. 2008), and is also affected by compounds targeting phospholipase C and PKC (Kelm et al. 2010). The potentiating effect of EtOH is impaired in central amygdala (CeA) in mice that lack PKCε (Bajo et al. 2008). Thus, PKC is implicated in both the pre and post-synaptic effects of EtOH at GABAergic synapses. It is notable that GABA release appears to be increased in the PKCε knockout mice prior to EtOH exposure, and thus the effect in this case may be more akin to occlusion rather than blockade of the drug action. It remains to be determined whether the effects of EtOH on these signaling molecules are direct or indirect.

In contrast to the effects on GABA release, the vast majority of studies indicate that acute EtOH either has no effect or inhibits release of glutamate (reviewed in Siggins et al. 2005, although see Xiao et al. 2009; Eggers and Berger 2004). These findings suggest a fundamental difference between GABAergic and glutamatergic terminals in most brain regions that may be useful in determining what factors contribute to EtOH sensitivity of release.

1.4 Monoamines and Neurotransmitter Transport

Acute EtOH effects on neurotransmitter transport have been investigated using brain tissue and heterologous expression systems. In vivo studies indicate that EtOH increases monoamine levels in brain (reviewed in Gonzales et al. 2004, LeMarquand et al. 1994; Thielen et al. 2001). However, most studies of neurotransmitter transporters show them to be relatively insensitive to EtOH. However, increased cell surface expression of the dopamine transporter (DAT) was observed when this protein was heterologously expressed (Mayfield et al. 2001; Maiya et al. 2002). This effect would most likely decrease striatal dopamine during acute in vivo EtOH exposure in rodents, and thus does not help to explain the findings from in vivo studies. However, there is some controversy as to whether EtOH has potent effects on dopamine uptake measured in brain tissue using voltammetric

techniques (Jones et al. 2006; Mathews et al. 2006; Robinson et al. 2005; Yavich and Tiihonen 2000). The EtOH-induced increase in striatal DA levels is unperturbed in DAT knockout mice, suggesting that the drug action responsible for this effect does not involve the transporter (Mathews et al. 2006). Furthermore, studies using in vitro voltammetry and in vivo microdialysis to measure dopamine levels indicate that direct infusion of EtOH into striatum does not alter DA levels (Mathews et al. 2006; Yan 2003; Yim et al. 1998). Thus, the physiologic impact of alterations in DAT function is not yet clear.

Examination of EtOH effects on the brain serotonergic system has yielded interesting findings. In addition to potentiating 5-HT_3 receptor function, as mentioned in the previous section on ligand-gated ion channels, inhibition of 5-HT1c by EtOH has also been reported (Sanna et al. 1994) although it is not clear whether this inhibition results from a direct effect on the receptor or on downstream signaling mechanisms. Exposure to acute EtOH also increases extracellular 5-HT levels in brain (LeMarquand et al. 1994; Thielen et al. 2001), and a recent report indicates that reduced 5-HT uptake may contribute to this effect as well as to the acute intoxicating effects of EtOH (Daws et al. 2006). However, EtOH effects on serotonin and other monoamines require further examination.

1.5 Ethanol and Synaptic Plasticity

Long-lasting changes in the efficacy of synaptic transmission are thought to contribute to brain development, learning and memory, and addiction (Hyman et al. 2006; Kauer and Malenka 2007). The most commonly studied forms of long-lasting synaptic plasticity are long-term potentiation (LTP), a persistent increase in synaptic transmission, and long-term depression (LTD), a persistent decrease in transmission. These types of plasticity are usually brought about by repetitive patterned activation of afferent inputs to a given postsynaptic neuron.

Effects of EtOH on LTP have been studied in different brain regions, but the majority of information comes from studies of the Schaffer collateral inputs to the CA1 pyramidal neurons of the hippocampal formation (Blitzer et al. 1990; Morrisett and Swartzwelder 1993; Mulkeen et al. 1987; Sinclair and Lo 1986). Acute EtOH exposure generally suppresses the induction of LTP at this and other synapses (Yin et al. 2007; Blitzer et al. 1990; Givens and McMahon 1995; Morrisett and Swartzwelder 1993; Mulkeen et al. 1987; Sinclair and Lo 1986; Wayner et al. 1993; Weitlauf et al. 2004). Effects occur at EtOH concentrations associated with intoxication, and in some studies at surprisingly low concentrations (Blitzer et al. 1990; Fujii et al. 2008). EtOH also inhibits LTP induced by kainate receptor activation in the basolateral amygdala (Lack et al. 2008).

There is not as much information regarding EtOH effects on LTD. Two prominent subtypes of LTD can be elicited in the hippocampal CA1 region. The most widely studied form of LTD is induced by repetitive low-frequency synaptic activation, and requires activation of NMDA receptors (Dudek and Bear 1992;

Mulkey and Malenka 1992). In the hippocampal CA1 region LTD is enhanced by exposure to EtOH at a concentration associated with strong intoxication (Hendricson et al. 2002), although this observation has not been consistent (Izumi et al. 2005).

Other forms of LTD observed in hippocampus and elsewhere involve activation of mGluRs (reviewed in Lüscher and Huber 2010). One report indicates that EtOH, at concentrations associated with severe intoxication, prevents mGluR-LTD at hippocampal synapses (Overstreet et al. 1997). At glutamatergic synapses onto cerebellar Purkinje neurons mGluR-LTD involves decreased surface expression and function of AMPARs (Ito 2001). Acute EtOH exposure inhibits this cerebellar LTD (Belmeguenai et al. 2008; Su et al. 2010), most likely due to inhibition of voltage-gated calcium channels and mGluR function. This finding is intriguing given that acute EtOH is known to impair motor coordination, and cerebellar function has been implicated in these effects. In the dorsal striatum, LTD involving these receptors also requires endocannabinoid (EC) signaling from the post to the presynaptic neuron (retrograde EC signaling) and subsequent activation of CB1 cannabinoid receptors (Gerdeman et al. 2002). The expression of this form of LTD appears to be on the presynaptic side of the synapse. Acute EtOH increases the expression of this EC-dependent mGluR-LTD in dorsal striatum (Yin et al. 2007). It is presently not clear what mechanisms contribute to this effect of EtOH.

2 Chronic Ethanol Actions

2.1 Chronic Ethanol Effects on Glutamatergic Transmission and Glutamate Roles in Synaptic Plasticity

Chronic EtOH treatment in animals provides critical information relevant to central changes that take place during long-term alcohol abuse in humans. Persistent EtOH exposure produces both tolerance and dependence. Tolerance is manifested as a decreased behavioral response to EtOH that implies a decrease in the intoxicating effects and other responses to the drug. Therefore, higher amounts of EtOH are required to achieve the same intoxicating effects seen with acute drug administration. EtOH dependence is generally described by symptomology elicited during and following withdrawal from EtOH (Heilig et al. 2010). These effects include anxiety, dysphoria and increased seizure susceptibility, hyperalgesia and disruption of sleep states (Enoch 2008; Grobin et al. 1998; Kumar et al. 2009). Chronic EtOH treatment is known to induce many neuroadaptative changes in the CNS involving both glutamatergic and GABAergic synaptic transmission.

The majority of work on chronic EtOH effects on glutamatergic transmission has focused on changes in glutamate receptors, particularly in light of the sensitivity of these receptors to acute EtOH actions (see previous discussion). Chronic EtOH exposure generally produces an increase in the function of NMDARs and in

NMDAR-mediated glutamatergic synaptic transmission (Cebere et al. 1999; Grover et al. 1998; Gulya et al. 1991; Lack et al. 2007; Smothers et al. 1997) (Fig. 1d). Initial studies examined effects of receptor activation on neuronal calcium and nitric oxide signals either in preparations made from EtOH-exposed animals or in cultured neurons treated with EtOH in the medium (Grover et al. 1998; Gulya et al. 1991; Chandler et al. 1997; Iorio et al. 1992; Smothers et al. 1997). Exposure to EtOH for days to weeks increased NMDAR agonist-induced increases in intracellular calcium. These effects could be observed at EtOH concentrations that did not alter neuronal viability and did not affect baseline intracellular calcium levels. Furthermore, changes in responses to NMDAR activation were consistently larger than changes in the effects of activation of other ionotropic glutamate receptors (Chandler et al. 1997; Gulya et al. 1991; Smothers et al. 1997). Direct examination of ion current through the NMDAR pore has revealed effects consistent with a chronic EtOH-induced upregulation of NMDAR function (Floyd et al. 2003; Grover et al. 1998). An increase in the component of current mediated by NR2B-containing receptors has also been observed (Floyd et al. 2003; Kash et al. 2009; Roberto et al. 2004b, 2006). Interestingly, acute EtOH inhibition of NMDARs in most brain regions is still intact or even increased after chronic in vivo exposure (Floyd et al. 2003; Roberto et al. 2006; Roberto et al. 2004b), although a small decrease in inhibition was observed in medial septum/ diagonal band neurons (Grover et al. 1998). Evidence of tolerance to EtOH inhibition during acute exposure has also been observed in hippocampal slices (Grover et al. 1994; Miyakawa et al. 1997). Overall, it appears that NMDAR function is still suppressed during intoxication even after prolonged EtOH exposure, and thus the increase in NMDAR function is likely to be dramatic after EtOH withdrawal following chronic exposure. One consequence of the increase in NMDAR-mediated calcium influx appears to be an increase in susceptibility to excitotoxic effects of NMDA (Chandler et al. 1993; Iorio et al. 1993), although enhanced NMDAR-mediated neuroprotection can also be observed in young cerebellar granule neurons (Pantazis et al. 1998). It has thus been postulated that excitotoxicity during EtOH withdrawal contributes to alcohol-related neuronal loss in the brain.

The mechanisms underlying the increase in NMDAR function are still under investigation, but several interesting facets of the story have already emerged. Analysis of receptor function and pharmacology, as well as examination of receptor subunit expression and location, indicate that receptors containing the NR2B subunit are the subtypes most strongly affected by chronic EtOH exposure (Carpenter-Hyland et al. 2004; Floyd et al. 2003; Kash et al. 2009; Roberto et al. 2004b) (Fig. 1d). The molecular basis of increased NR2B function is less clear. While some investigators have reported increases in NR2B mRNA expression following chronic alcohol exposure in vitro (Hu et al. 1996; Snell et al. 1996), and in vivo (Follesa and Ticku 1995; Kash et al. 2009; Roberto et al. 2006) such increases have not been observed in every brain region (Cebere et al. 1999; Floyd et al. 2003; Läck et al. 2005). Increases in NR2B, and to a lesser extent NR2A, protein expression have also been observed using immunologic techniques after both in vitro and in vivo EtOH exposure (Kash et al. 2005; Obara et al. 2009;

Snell et al. 1996). However, other investigators did not observe increased expression of this protein. Increased expression of mRNA and protein for other NR subunits and particular NR1 splice variants has been observed in some brain regions following chronic EtOH exposure (Raeder et al. 2008; Trevisan et al. 1994; Roberto et al. 2006; Winkler et al. 1999, but see Morrow et al. 1994), but there is less evidence for increased receptor function as a result of these increases. Thus, it is not clear whether increased subunit expression is the driving force behind increased receptor function, and if so, what mechanisms underlie the increase in expression or trafficking.

Changes in subcellular distribution of receptors may also contribute to altered NMDAR function following chronic EtOH exposure. In cultured hippocampal neurons, exposure to EtOH leads to increased NMDAR expression in dendritic spines, the location of glutamatergic synapses (Carpenter-Hyland et al. 2004). This increased trafficking to spines is accompanied by an increase in the contribution of NMDARs to glutamatergic transmission, but does not appear to involve increased NMDAR protein expression. The synaptic NMDARs observed following chronic EtOH exposure appear to contain the NR2B subunit. Increases in the contribution of NMDARs to glutamatergic synaptic transmission have also been observed following subacute (10 s of seconds or minutes) EtOH exposure, and NR2B-containing receptors also appear to contribute to these increases (Wang et al. 2007; Yaka et al. 2003). Tyrosine phosphorylation by a Fyn-like kinase has been implicated in these rapid increases in the function of NR2B-containing receptors (Wang et al. 2007), but it is yet to be determined whether this mechanism plays a role in chronic EtOH effects on the receptor.

Chronic EtOH effects on AMPA and kainate receptors have been examined, with variable results. Increases in AMPA receptor subunit mRNA have been observed in hippocampus following chronic EtOH exposure (Bruckner et al. 1997). Expression of AMPAR subunit proteins was also induced by chronic exposure in primary cortical cultures (Chandler et al. 1999), while increased AMPAR binding was observed in cortical membranes from EtOH exposed animals (Haugbol et al. 2005). Evidence of increased AMPAR function has also been reported following chronic EtOH exposure, as measured with intracellular calcium signals in cerebellar Purkinje neurons (Netzeband et al. 1999), and AMPA receptor-mediated synaptic responses are increased in basolateral amygdala (Lack et al. 2007). This latter effect was observed following during withdrawal but not just after the end of chronic EtOH exposure. However, other studies have reported that AMPAR expression and function are not altered following chronic EtOH exposure (e.g. Smothers et al. 1997). The factors that underlie this variability in findings may include the type of preparation examined, the duration and pattern of EtOH exposure, and whether assays were performed just after the end of drug exposure or after withdrawal had been allowed to proceed. With respect to kainate receptors, Chandler and collaborations (Chandler et al. 1999) observed no change in receptor expression in cultured cortical neurons following chronic EtOH exposure. In contrast, enhancement of both subunit protein and kainate receptor function was found in cultured hippocampal neurons (Carta et al. 2002), and

chronic intermittent EtOH increased KAR-mediated synaptic transmission in basolateral amygdala (Lack et al. 2009).

Chronic EtOH intake has also been shown to enhance intracellular signaling associated with mGluRs, particularly mGluR5, in the nucleus accumbens (NAc) (Cozzoli et al. 2009). While chronic EtOH drinking can induce increases in mGluR1 and mGluR5 protein expression in NAc and amygdala (Szumlinski et al. 2008; Obara et al. 2009), changes in mGluR5 signaling in NAc are not always associated with an increase in the protein itself (Szumlinski et al. 2008). In cultured cerebellar Purkinje neurons, exposure to EtOH for 11 days produced a decrease in mGluR-induced dendritic calcium signals (Netzeband et al. 2002). Clearly, more work is needed to determine how signaling by the many mGluR subtypes changes with long-term EtOH exposure and drinking.

Measurements of extracellular glutamate levels in brain have generally shown increases produced by chronic EtOH exposure, especially after withdrawal or repeated cycles of withdrawal (Dahchour and De Witte 1999, 2003; Rossetti and Carboni 1995; Roberto et al. 2004b). These findings have generally been derived from measurements using in vivo microdialysis in brain. However, microdialysis measures of this type must be interpreted carefully, as both synaptic and nonsynaptic sources of glutamate contribute to the extracellular pool of this amino acid. Indeed, there is mounting evidence that changes in the cystine/glutamate exchanger generate increases in extracellular glutamate produced by some drugs of abuse (Kalivas 2009). Evidence of increased synaptic glutamate release has been observed in amygdala following chronic EtOH treatment (Lack et al. 2007; Zhu et al. 2007; Roberto et al. 2004b). Decreases in glutamate uptake have also been noted following chronic EtOH exposure (Melendez et al. 2005). There may be multiple factors that contribute to increased extracellular glutamate levels and increased glutamatergic transmission following chronic EtOH exposure and withdrawal.

Despite the evidence that NMDAR function and extracellular glutamate levels are increased following chronic EtOH exposure, studies of hippocampal LTP indicate that this form of synaptic plasticity is decreased under the same conditions (Durand and Carlen 1984; Roberto et al. 2002, although see Fujii et al. 2008). Similar results have been obtained in the amygdala (Stephens et al. 2005). It is not yet clear what factors underlie the decrease in LTP, but it is most likely that the loss of plasticity involves mechanisms occurring downstream of NMDAR activation in the LTP induction process.

2.2 *Chronic Ethanol and GABAergic Transmission: Postsynaptic Effects*

Chronic EtOH treatment is known to induce many neuroadaptative changes in the CNS. Over the past 20 years, it has been widely demonstrated that GABAergic transmission is sensitive to EtOH in distinct brain regions and is clearly involved

in EtOH tolerance and dependence (Eckardt et al. 1998; Grobin et al. 1998). Chronic EtOH exposure often results in the development of tolerance to many GABAergic effects of the drug including the anxiolytic, sedative, ataxic, and positive reinforcing effects (Kumar et al. 2004, 2009). Substantial evidence suggests that these behavioral and neural adaptations involve marked changes in the expression profile of specific $GABA_A$ receptor subunits (Grobin et al. 1998) and in the pharmacological properties of $GABA_A$ receptors (Kang et al. 1998b) (Fig. 1).

Chronic EtOH administration differentially altered the expression of distinct $GABA_A$ receptor subunit mRNAs and peptide levels in various brain regions. In the cerebral cortex, both mRNA and peptide levels for $GABA_A$ receptor $\alpha1$, $\alpha2$, and $\alpha3$ subunits were decreased (Devaud et al. 1995, 1997). In contrast, both $\alpha4$, $\beta1$, $\beta2$, $\beta3$, $\gamma1$ and $\gamma2$ subunit mRNA and peptide levels were increased (Devaud et al. 1995, 1997). These alterations in the subunit expression affect the $GABA_A$ receptor assemblage and consequently, also affect receptor function and binding. It has been reported that recombinant $GABA_A$ receptors with $\alpha4\beta2\gamma2$ subunits are less sensitive to GABA and benzodiazepines compared to $\alpha1\beta2\gamma2$ receptors (Whittemore et al. 1996). Therefore, these alterations may account for the decreased sensitivity to GABA in cerebral cortical synaptoneurosomes (Morrow et al. 1988) and benzodiazepines in cortical membrane vesicles (microsacs) (Buck and Harris 1990). Following chronic EtOH exposure, acute EtOH did not facilitate the GABA or muscimol-stimulated Cl- uptake in cortex (Morrow et al. 1988) and in cerebellum (Allan and Harris 1987). In the cerebellum, chronic EtOH exposure decreased $GABA_A$ receptor $\alpha1$ subunit mRNA and increased $\alpha6$ subunit mRNA (Mhatre and Ticku 1992; Morrow et al. 1992). Chronic EtOH administration also decreased the polypeptide levels of the δ subunit of $GABA_A$ receptors in the rat cerebellum and hippocampus, whereas there were no changes in the δ subunit polypeptide levels in the rat cerebral cortex (Marutha Ravindran et al. 2007). Furthermore, chronic EtOH administration caused a down-regulation of native δ subunit-containing $GABA_A$ receptor assemblies in the rat cerebellum as determined by [(3)H]muscimol binding to the immunoprecipitated receptor assemblies (Marutha Ravindran et al. 2007).

The alterations in $GABA_A$ receptor gene expression are regionally and temporally dependent. For example, chronic EtOH consumption produced a significant increase in the level of $GABA_A$ receptor $\alpha4$ subunit peptide in the hippocampus following 40 days but not 14 days exposure (Matthews et al. 1998). The relative expression of hippocampal $GABA_A$ receptor $\alpha1$, $\alpha2$, $\alpha3$, β(2/3), or $\gamma2$ subunits was not altered by either period of chronic EtOH exposure (Charlton et al. 1997; Matthews et al. 1998). Hippocampal $\alpha1$ subunit immunoreactivity and mRNA content were also significantly reduced after 12 weeks of treatment, but not after 4 weeks of exposure. In contrast, $\alpha5$ mRNA content was increased in this brain region. In marked contrast, chronic EtOH consumption for both 14 (Devaud et al. 1997) and 40 (Devaud et al. 1997; Matthews et al. 1998) days significantly increased the relative expression of cerebral cortical $GABA_A$ receptor $\alpha4$ subunits and significantly decreased the relative expression of $\alpha1$ subunits (Devaud et al. 1997; Matthews et al. 1998). These findings indicate that chronic EtOH

consumption alters $GABA_A$ receptor gene expression in the hippocampus but in a different manner from that in either the cerebral cortex or the cerebellum. In addition, these alterations are dependent on the duration of EtOH exposure (Grobin et al. 1998).

The Olsen and Spigelman groups have developed a chronic intermittent EtOH treatment paradigm in which rats are given a 5–6 g/kg dose of EtOH on alternate days for 60 treatments (120 days). This chronic administration of EtOH to rats on an intermittent regimen, for 60 repeated intoxicating doses and repeated withdrawal episodes, increases levels of $\alpha 4$ subunit mRNA in hippocampus with no significant change in the mRNAs for the $\alpha 5$ subunit (Mahmoudi et al. 1997). Similarly, rats that were exposed to intermittent episodes of intoxicating EtOH and withdrawal showed increased hippocampal $\alpha 4$ subunit peptide expression (Cagetti et al. 2003) and alteration in the pharmacological responses of $GABA_A$ receptors to benzodiazepine agonists and inverse agonists (Cagetti et al. 2003). The mRNA levels for the $\gamma 2S$ and $\gamma 1$ subunits were also elevated. In CA1 pyramidal slices from chronic intermittent EtOH exposed rats, the baseline decay time of $GABA_A$R-mediated mIPSCs was decreased, and the positive GABA receptor modulation of mIPSCs was also reduced compared with control rats. However, mIPSC potentiation by the α-preferring benzodiazepine ligand bretazenil was maintained, and mIPSC potentiation by Ro15-4513 was increased (Cagetti et al. 2003; Liang et al. 2009).

In the VTA, levels of $\alpha 1$ subunit immunoreactivity were significantly decreased after 12 weeks but not 1–4 weeks of treatment (Charlton et al. 1997). Papadeas et al. (2001) found that in the amygdala, $\alpha 1$ and $\alpha 4$ subunit expression was significantly decreased after two weeks of chronic EtOH consumption. In the nucleus accumbens (NAC), $\alpha 4$ subunit expression was decreased, but $\alpha 1$ subunit expression was not altered. In the VTA, there were no changes in $\alpha 1$ and $\alpha 4$ subunit expressions. Muscimol-stimulated Cl^- uptake was enhanced in the extended amygdala, but not the NAC of EtOH-dependent rats. These results suggest that chronic EtOH exposure alters $GABA_A$ receptor expression in the amygdala and NAC and that decreased expression of $\alpha 4$ subunits is associated with increases in $GABA_A$ receptor function in the amygdala but not the NAC (Papadeas et al. 2001).

Alterations in subunit assembly could induce alterations in the functional properties of $GABA_A$ receptors without alterations in the total number of receptors (Devaud et al. 1995; Kumar et al. 2009; Morrow et al. 1992). The expression of $GABA_A$ receptors involves a highly regulated process of synthesis, assembly, endocytosis, and recycling or degradation. Changes in the expression and composition of various $GABA_A$ receptors could result from selective endocytosis, recycling, and/or trafficking of newly synthesized receptors to the cell surface. $GABA_A$ receptor trafficking on the cell surface following EtOH consumption is thought to contribute to the development of EtOH-dependence (Kumar et al. 2004). It has been reported by Kumar et al. (2003) that chronic EtOH exposure selectively increases the internalization of $\alpha 1$ $GABA_A$ receptors with no change in the internalization of $\alpha 4$ $GABA_A$ receptors into clathrin coated vesicles of the cerebral cortex. There is also a decrease in $\alpha 1$ $GABA_A$ receptors and a

significant increase in $\alpha 4$ subunit peptide in the synaptic fraction following chronic EtOH exposure. These results suggest that the regulation of intracellular trafficking following chronic EtOH administration may alter the subtypes of $GABA_A$ receptors on the cell surface and may account for changes in the pharmacological properties of $GABA_A$ receptors (Kumar et al. 2004) (Fig. 1).

Clathrin and the adaptor complex (AP) play a crucial role in the internalization of $GABA_A$ receptors following chronic EtOH administration. Notably, in the intracellular fraction, the clathrin-$\alpha 1$-$GABA_A$ receptor complex is increased following chronic EtOH administration (Kumar et al. 2004). Specific $GABA_A$ receptor subunits ($\beta 2$ and/or $\gamma 2$) are required for recognition of the receptor by the AP-2 that precedes clathrin-dependent endocytosis (Herring et al. 2003; Kittler et al. 2008). Chronic EtOH exposure induces an increase in the expression of $\alpha 4$-, $\beta 2$-, and $\beta 3$- $GABA_A$ receptor subunits in the cerebral cortex and all of these subunits contain consensus phosphorylation sites for PKC. In contrast, $\alpha 1$, $\alpha 2$, and $\alpha 3$ $GABA_A$ receptor subunits are decreased in the cortex and these subunits do not contain consensus phosphorylation sites for PKC. Hence, it has been hypothesized that PKC may phosphorylate the $GABA_A$ receptor subunits and/or AP-2 following chronic EtOH administration, altering the recognition and endocytosis of $GABA_A$ receptors by blocking AP-2 binding (Macdonald 1995; Mohler et al. 1996). A single dose of EtOH also increases the internalization of $GABA_A$ receptor $\alpha 4$ and δ subunits (Liang et al. 2007). In rat hippocampus, chronic EtOH exposure induces a decrease in the tyrosine kinase phosphorylation of $\alpha 1$ subunits, an increase of $\beta 2$ subunits and no alteration in $\gamma 2$ subunits (Marutha Ravindran et al. 2007).

$GABA_A$ receptor trafficking is regulated by many protein kinases, including PKC, PKA, and fyn. However, to date, the role of these protein kinases has not yet been studied in the trafficking of $GABA_A$ receptors, especially following EtOH exposure. Chronic EtOH consumption decreases association of PKCγ with $\alpha 1$ $GABA_A$ receptors and increases association of PKCγ with $\alpha 4$ $GABA_A$ receptors, accompanied by a decreased expression of the $\alpha 1$ subunit and an increased expression of $\alpha 4$ at the cell surface in cerebral cortex (Kumar et al. 2002). However, there were no alterations in the association of PKCγ with $GABA_A$ receptors in the $\alpha 1$ subunit expression following chronic EtOH administration in the hippocampus (Kumar et al. 2004). The increased association of PKCγ with $\alpha 4$ $GABA_A$ receptors may phosphorylate $GABA_A$ receptor subunits and prevent recognition of the receptor by AP-2, thus preventing its internalization. Indeed, phosphorylation of $GABA_A$ receptor subunits reduced the binding of receptors with AP-2 and subsequent internalization (Kittler et al. 2008). Moreover, reduced PKC-dependent $GABA_A$ receptor phosphorylation increases receptor binding to the AP-2 and promotes receptor endocytosis (Terunuma et al. 2008). Chronic activation of PKA in cerebellar granule cells increases cell surface expression of $GABA_A$ receptor $\alpha 1$ subunit (Ives et al. 2002). EtOH exposure alters expression and translocation of PKA (Diamond and Gordon 1994; Newton and Messing 2006) suggesting that PKA is likely also involved in the trafficking of $GABA_A$ receptors following EtOH exposure. Future studies will determine the specific role of

various protein kinases in $GABA_A$ receptor trafficking following chronic EtOH administration.

Post-translational modifications such as phosphorylation and glycosylation of $GABA_A$ receptors may play a role in the development of EtOH-dependence. In particular, phosphorylation of $GABA_A$ receptors has been demonstrated to modulate receptor function. In *Xenopus* oocytes and isolated mouse brain membrane vesicles (microsacs), PKC and PKA phosphorylation of $GABA_A$ receptors decreases receptor activation (Kellenberger et al. 1992; Krishek et al. 1994; Leidenheimer et al. 1992). Phosphorylation by CAM kinase II or tyrosine kinase enhances $GABA_A$ receptor function (Churn et al. 2002; Valenzuela et al. 1995). As discussed previously, acute EtOH induces changes in $GABA_A$ receptor function that may be dependent on phosphorylation of particular proteins. Chronic EtOH exposure might be expected to result in long-term changes in second messenger systems, including kinase activity. However, the heterogeneity of $GABA_A$ receptors expressed in vivo has precluded definitively answering this question and none of these studies have directly demonstrated that phosphorylation is involved in EtOH modulation of $GABA_A$ receptor function. The exact mechanisms involved in the alteration of $GABA_A$ receptor function following chronic EtOH exposure still remain to be determined.

From the preceding review, it is clear that the majority of the early studies characterizing chronic effects of EtOH on GABAergic transmission focused mainly on postsynaptic properties and the subunit composition of the $GABA_A$ receptors themselves. Some of the disparity in the findings across laboratories on postsynaptic sites of EtOH action may reflect the differences in the chronic EtOH treatment duration and protocol, brain region examined, and methods of assessing receptor function. Most of these studies were generally in agreement that chronic EtOH exposure and withdrawal did not result in dramatic decreases in the number of $GABA_A$ receptors in most brain regions. However, many of these studies reported marked alterations in the expression of specific $GABA_A$ receptor subunits and hypothesized that those changes in the subunit composition of the $GABA_A$ receptors may account for the physiologic and pharmacologic alterations in GABAergic signaling associated with chronic EtOH administration (Grobin et al. 1998).

Of particular clinical importance is the development of tolerance and dependence to EtOH, and it is likely that adaptive changes in synaptic function in response to ethanol's actions on $GABA_A$ receptors play a role in this process. Indeed, it is well known that chronic EtOH treatment can lead to tolerance and physical dependence (Chandler et al. 1998) and withdrawal following long-term EtOH consumption is associated with increased neuronal excitability (Kliethermes 2005; Weiner and Valenzuela 2006). These alterations have been hypothesized to represent, in part, a compensatory adaptation to the in vitro acute facilitatory effects of EtOH on GABAergic synapses (Siggins et al. 2005; Weiner and Valenzuela 2006). Few studies have reported the effects of long-term EtOH exposure on GABAergic synaptic transmission looking at both postsynaptic and presynaptic mechanisms using in vitro brain slice methods.

As described above, the adaptive changes in $GABA_A$ receptor expression are thought to lead to a pronounced hypofunction of GABAergic neurotransmission and possibly the development of tolerance to the in vitro acute effects of EtOH on these synapses. In the hippocampus, there is a decrease in the threshold for seizure induction by the $GABA_A$ receptor antagonist pentylenetetrazole (Kokka et al. 1993) and a decrease in $GABA_A$ receptor activity in hippocampal slices that also lasts for at least 40 days after the last EtOH dose (Cagetti et al. 2003; Kang et al. 1996; Liang et al. 2004, 2009). Using analysis of tetrodotoxin (TTX)-resistant mIPSCs recorded from CA1 pyramidal neurons of chronic EtOH exposed and control rats, this group demonstrated a significant decrease in the amplitude and decay of these responses (Cagetti et al. 2003) possibly reflecting the observed alteration in the expression of $\alpha 1$ and $\alpha 4$ subunits. The mIPSC frequency is also slightly decreased, suggesting that chronic EtOH exposure may also be associated with a presynaptic decrease in GABA release at these synapses (see later section). Importantly, the pharmacological alterations in the properties of GABAergic synapses were consistent with the observed changes in subunit expression. For example, diazepam and the neurosteroid alphaxalone did not have any effect on mIPSCs in slices from chronic EtOH exposed rats (Cagetti et al. 2003), possibly reflecting the loss of $\alpha 1$ and γ-subunits, respectively.

On the other hand, drugs with some selectivity for $\alpha 4$-subunits (e.g., RO 15-4513 and DMCM) showed an increased modulation of mIPSCs possibly reflecting the increase in $\alpha 4$ subunit expression (Kang et al. 1996, 1998a, b). Interestingly, the evoked IPSCs were still sensitive to alphaxalone (Kang et al. 1998b) suggesting differences in the populations of $GABA_A$ receptors that underlie evoked and mIPSCs. In addition, the acute effect of EtOH on evoked IPSCs was significantly increased in slices from chronic EtOH-exposed rats (Kang et al. 1998a, b). Liang et al. (2004) have also compared the effects of chronic EtOH exposure on synaptic and extra-synaptic receptor functions in CA1 neurons. These investigators found similar alterations in the synaptic mIPSCs and the tonic extra-synaptic $GABA_A$ receptor-mediated conductance associated with chronic EtOH exposure. Both mIPSCs and the tonic current show profound tolerance to $\alpha 1$-containing $GABA_A$ receptor selective doses of diazepam and zolpidem (Cagetti et al. 2003). As previously demonstrated (Grobin et al. 2000), chronic EtOH exposure results in a decrease in BZP-sensitive $\alpha 1$-subunits and an increase in BZP-insensitive $\alpha 4$-subunits at synaptic receptors. Thus, THIP (a high affinity and efficacy agonist of the $\alpha 4$-containing $GABA_A$ receptors and a partial agonist at most other $GABA_A$ receptor assemblies) activated the tonic GABA current in slices from control-untreated rats and had little effect in slices from chronic EtOH exposed rats (Liang et al. 2004). However, THIP depressed mIPSCs in control-untreated rats but strongly increased mIPSCs in chronic EtOH-treated rats. In addition, the chronic EtOH-treated rats show a modest tolerance to the soporific effects of THIP and no change in its anxiolytic effects (Liang et al. 2004).

In the previous decade, non-human primates (*Cynomolgus macaques*) have been a powerful model to study the effects of long-term EtOH consumption (Vivian et al. 2001). Ongoing research in the Weiner lab has provided the first

evidence of neuroadaptations in the GABAergic synapses in monkey hippocampus (Weiner et al. 2005). In this paradigm of EtOH self-administration, cynomolgus macaques are trained to self administer a 4% EtOH solution on an operant panel and then given 22 h daily access to the EtOH solution. Control subjects were age- and sex-matched animals that had free access to food and water but were not exposed to the operant panels. The preliminary in vitro electrophysiologic findings revealed a significant increase in paired-pulse facilitation (PPF) of $GABA_A$ IPSCs in dentate granule cells in slices prepared immediately following the last day of 18 months of daily EtOH drinking. Their finding is consistent with a decrease in release probability (see later section) and is in agreement with the decrease in mIPSC frequency observed in rats following chronic intermittent EtOH exposure (Cagetti et al. 2003). Interestingly, there was lack of tolerance for both the acute facilitatory effect of EtOH and flunitrazepam on evoked $GABA_A$ IPSCs (Weiner et al. 2005). Using the same paradigm of EtOH self-administration, whole-cell patch clamp recordings on acutely dissociated amygdala neurons from EtOH-exposed cynomolgus macaques showed a decrease in the effect of flunitrazepam on the currents gated by exogenous GABA application compared with amygdala neurons from control animals (Anderson et al. 2007; Floyd et al. 2004). However, the modest inhibition of GABA-gated currents induced by acute EtOH was not affected by the chronic EtOH consumption. In addition, mRNA expression levels for the β, γ, and δ subunits in total amygdala RNA isolated from control and EtOH-drinking animals were measured. Chronic EtOH significantly reduced amygdala $\beta1$ and $\gamma2$ subunit expression. Overall, these findings demonstrate that chronic EtOH self-administration reduces the benzodiazepine sensitivity of amygdala $GABA_A$ receptors and this reduced sensitivity may reflect decreased expression of the γ subunit.

Roberto et al. (2004a) recently assessed whether GABAergic synaptic changes occur with EtOH-dependence in rat central amygdala (CeA) slices. To obtain dependent rats, these investigators used an EtOH vapor inhalation method (Rogers et al. 1979). In this study, male Sprague–Dawley rats were exposed to a continuous EtOH vapor for 2–3 weeks with a targeted blood alcohol level of 150–200 mg/dL. Control rats were maintained in similar chambers without EtOH vapor. On experiment days, the chronic EtOH-treated rats were maintained in the EtOH vapor chamber until preparation of the CeA slices, and recordings of GABAergic transmission were made in EtOH-free solution 2–8 h after cutting the slices (Roberto et al. 2004a). The evoked IPSCs in CeA neurons from EtOH-dependent rats were significantly larger than in naïve rats. In EtOH-dependent rats, the mean baseline amplitude of mIPSCs was also significantly increased compared to naïve rats, suggesting a post synaptic effect of chronic EtOH (Roberto et al. 2004a). However, possible changes in the expression of $GABA_A$ receptor subunits were not characterized. It was also found that the baseline PPF ratio of IPSCs was significantly decreased and the mIPSC frequency was higher in neurons of EtOH-dependent rats compared to naïve rats, suggesting that GABA release was augmented in chronic EtOH treated rats (Roberto et al. 2004a) (see later section on presynaptic change).

In addition, acute EtOH (44 mM) increased IPSCs, decreased the PPF ratio of IPSCs and increased the mIPSCs frequency to the same extent in EtOH-dependent rats and naïve rats, suggesting a lack of tolerance for the acute EtOH effects (Roberto et al. 2004a). One of the most consistent findings from these recent studies is the lack of tolerance for the acute potentiating effect of EtOH on GABAergic synapses. These studies suggest that GABAergic mechanisms may not be associated with the tolerance that is known to develop with some of the behavioral effects of EtOH (e.g. ataxia, sedation). Additional studies will be needed to more carefully determine the molecular mechanisms responsible for these adaptive changes in different brain regions and length/duration of EtOH exposure required to induce such neuroadaptations in GABAergic synapse. Moreover, these data also suggest that, as with the acute effects of EtOH, long-term exposure to EtOH results in both pre and postsynaptic alterations and these changes may differ between brain regions (Siggins et al. 2005; Weiner and Valenzuela 2006).

2.3 Chronic Ethanol and GABAergic Transmission: Presynaptic Effects

There are only a few studies reporting that chronic EtOH exposure can alter GABAergic transmission by effects on GABA release. Short in vitro chronic EtOH exposure (one day) induced a transient decrease in mIPSC duration in cultured cortical neurons. Chronic EtOH exposure did not change mIPSC frequency nor did it produce a substantial cross-tolerance to a benzodiazepine in cortical neurons (Fleming et al. 2009). The results suggest that EtOH exposure in vitro has limited effects on synaptic $GABA_AR$ function and action potential-independent GABA release in cultured neurons. This group also investigated the effect of chronic EtOH exposure on GABA release in cultured hippocampal neurons (Fleming et al. 2009). These investigators found that chronic EtOH exposure did not alter mIPSC kinetics and frequencies in hippocampal neurons (Fleming et al. 2009). These results suggest that EtOH exposure in cultured cortical and hippocampal neurons may not reproduce all the effects that occur in vivo and in acute brain slices.

In fact, more results generated using in vitro brain slices show a stronger effect of EtOH on GABA release, as discussed earlier in this review (Fig. 1). In vitro brain slice preparations provide a number of highly sensitive experimental strategies that can be employed to detect presynaptic changes in transmitter release (for reviews of these approaches, see Siggins et al. 2005; Weiner and Valenzuela 2006).

Studies in the hippocampus show that chronic EtOH exposure decreased long-term potentiation (LTP) by increasing the electrically stimulated (but not basal) release of tritiated GABA pre-loaded in CA1 hippocampal slices (Tremwel et al. 1994). The GABA uptake or $GABA_AR$ function was not altered, and this effect may be due to alterations in the muscarinic receptor regulation of GABA release at

presynaptic terminals (Hu et al. 1999). In addition, studies using the $GABA_B$ receptor agonist baclofen to reduce release of tritiated GABA suggest that a change in $GABA_B$ auto-receptors on GABAergic terminals may also contribute to this effect of chronic EtOH exposure on LTP (Peris et al. 1997) (see later $GABA_B$ paragraph). For a general review of brain region specific EtOH actions on the GABA system see (Criswell and Breese 2005; Siggins et al. 2005; Weiner and Valenzuela 2006). More recent studies also reported that chronic EtOH consumption induces tolerance to the impairing effects of acute EtOH treatment on induction of LTP in rat CA1 slices (Fujii et al. 2008). In CA1 slices from control rats, stable LTP was induced by tetanic stimulation, and LTP induction was blocked if the tetanus was delivered in the presence of 8.6 mM EtOH or muscimol. A decrease in the stimulation threshold for inducing LTP was found in hippocampal slices from chronic EtOH-treated rats. In addition, application of EtOH or muscimol did not affect LTP induction in these cells, suggesting that the effects of chronic EtOH exposure on LTP induction are mediated by a reduction in GABAergic inhibition in hippocampal CA1 neurons (Fujii et al. 2008).

Weiner et al. (2004) found that voluntary EtOH drinking is associated with a significant increase in paired-pulse plasticity at GABAergic synapses in dentate gyrus neurons from the hippocampal formation of monkeys (cynomolgus macaques), consistent with a reduction in GABA release probability. In addition, a lack of tolerance to the facilitating effects of both acute EtOH and flunitrazepam on the $GABA_A$ IPSCs was reported.

In contrast, Melis et al. (2002) reported that a single EtOH exposure in vivo induces a long-lasting facilitation of GABA transmission in the VTA of EtOH-preferring C57BL/6 mice. These investigators observed that evoked $GABA_A$ IPSCs in dopaminergic neurons of EtOH-treated animals exhibited paired-pulse depression (PPD) compared with saline-treated animals, which exhibited PPF (Melis et al. 2002). An increase in frequency of mIPSCs was also observed in the EtOH-treated animals. Moreover, the $GABA_B$ receptor antagonist, CGP35348, shifted PPD to PPF, indicating that presynaptic $GABA_B$ receptor activation, likely attributable to GABA spillover, might play a role in mediating PPD in the EtOH-treated mice (see later $GABA_B$ paragraph). In a more recent study, the same group (Wanat et al. 2009) demonstrated that EtOH exposure also increased GABA release onto VTA dopamine neurons in EtOH non-preferring DBA/2 mice. However, a single EtOH exposure reduced glutamatergic transmission and LTP in VTA dopamine neurons from the EtOH non-preferring DBA strain but not EtOH-preferring C57BL/6 mice (Wanat et al. 2009).

Additional data from Roberto et al. (2004a, 2010) further suggest that chronic EtOH exposure can affect CeA GABA release, perhaps via an action on GABAergic terminals. Baseline $GABA_A$ IPSCs were significantly higher, and baseline PPF of $GABA_A$ IPSCs was significantly smaller in CeA neurons from EtOH-dependent rats compared to non-dependent rats, suggesting that evoked GABA release was augmented after chronic EtOH exposure. These investigators also reported an increase in the baseline frequency of mIPSCs in CeA neurons from EtOH-dependent rats compared to that of naïve controls. Acute superfusion of EtOH significantly

enhanced $GABA_A$ IPSCs, decreased the PPF ratio of IPSCs ,and increased the mIPSC frequency to the same extent in CeA slices from EtOH-dependent rats and naïve rats, suggesting a lack of tolerance to the presynaptic acute EtOH effects (Roberto et al. 2004a). In addition, these investigators estimated the interstitial GABA levels in CeA using microdialysis in freely moving rats. In agreement with the in vitro electrophysiologic results, the in vivo data showed a fourfold increase of baseline dialysate GABA concentrations in CeA of EtOH-dependent rats compared to naïve rats. Moreover, local administration of EtOH by dialysis increased the dialysate GABA levels in CET rats. These findings again indicate a lack of tolerance to presynaptic acute EtOH effects on GABA release in CeA of CET rats (Roberto et al. 2004a). These studies strengthen the possibility that chronic as well as acute EtOH may alter the function of the GABAergic synapses acting at both the post-synaptic site and presynaptic terminals. Altogether, these data suggest that long-term exposure to EtOH causes changes at GABAergic synapses that may differ between brain regions and with the duration of chronic exposure. Further studies will be needed to more carefully determine the specific exposure durations required to elicit these changes in GABAergic synapses, the molecular mechanisms responsible for these adaptive changes, as well as their behavioral consequences with respect to withdrawal and dependence.

Another area in which action of EtOH on GABA function has been implicated is withdrawal from chronic EtOH. Withdrawal results in an increased sensitivity to induction of seizures (Allan and Harris 1987; Frye et al. 1983). Several functional and behavioral studies on benzodiazepines and other drugs with GABA mimetic action reduced such withdrawal-related hyper-excitability (Breese et al. 2006; McCown et al. 1985; Roberto et al. 2008; Ticku and Burch 1980). Collectively, these results offer strong support for the hypothesis that at least a part of the action of EtOH was mediated by effects on neural functions associated with GABA transmission and that these effects play an important role in the maintenance of addictive drinking behavior.

2.4 *γ-Aminobutyric Acid$_B$ Receptors and Chronic Ethanol Actions*

Several studies demonstrated $GABA_B$ receptor involvement in the effects of EtOH. For instance, $GABA_B$ receptor antagonists enhance the ability of acute EtOH to facilitate GABA transmission in the hippocampus (Ariwodola and Weiner 2004; Wan et al. 1996; Wu and Saggau 1994) and NAc (Nie et al. 2000). Ariwodola and Weiner (2004) suggested that the effect of EtOH to facilitate GABA transmission is limited because of GABA feedback on presynaptic $GABA_B$ receptors (Fig. 1). The presence of $GABA_B$ receptors accounted for the difference in sensitivity to EtOH influences on GABA transmission in specific subfields of the hippocampus (Weiner et al. 1997). On the other hand, $GABA_B$ receptors did not influence GABA release from neurons in the CeA (Roberto et al. 2003). Thus, the involvement of $GABA_B$ receptors on GABA release in various brain regions may

not be universal, suggesting that the presence or absence of presynaptic $GABA_B$ receptors may be an important determinant for the regional specificity of EtOH to affect GABA transmission (Ariwodola and Weiner 2004).

As mentioned above, Peris et al. (1997) showed that chronic EtOH treatment, sufficient for decreasing LTP in rats, also increased 3H-GABA release from hippocampal slices in these same animals. These investigators characterized presynaptic auto-receptor modulation of 3H-GABA release in hippocampal slices from control and EtOH-dependent rats. Effects of a $GABA_B$ receptor agonist (baclofen) and antagonist [2-hydroxy (OH)-saclofen] on electrically stimulated 3H-GABA release from superfused hippocampal slices were examined. Baclofen decreased stimulated release in a dose-dependent manner and the antagonist 2-OH-saclofen increased release consistent with the presence of presynaptic $GABA_B$ auto-receptors in hippocampus. The $GABA_A$ antagonist bicuculline did not significantly modulate basal or stimulated release. Presynaptic modulation of release by baclofen and 2-OH-saclofen was decreased in animals 48 h after withdrawal from EtOH. Using quantitative autoradiographic techniques, the density of 3H-baclofen binding sites in the hippocampus was not affected by chronic EtOH exposure, whereas the density of 3H-bicuculline binding sites was increased by 28% in EtOH-treated rats. These data may explain how chronic EtOH treatment increases presynaptic regulation of GABA release from hippocampus that may contribute to the decrease in LTP seen in rats after chronic EtOH exposure (Peris et al. 1997).

Another study assessed the impact of EtOH on postsynaptic $GABA_B$ receptors via baclofen-induced hyperpolarization of hippocampal CA1 and CA3 pyramidal neurons. These receptors activate outward K^+ currents via a pertussis toxin-sensitive G protein cascade to reduce membrane potential during the slow inhibitory postsynaptic potential and may play a role in EtOH intoxication and withdrawal excitability. In both types of pyramidal neurons, baclofen applied consecutively in increasing concentrations caused concentration-dependent hyperpolarization. There were no significant differences in resting membrane potential, input resistance, maximum baclofen-induced hyperpolarization, or EC_{50} between CA1 and CA3 neurons, although slope values were significantly smaller in the former neurons. These parameters were not significantly changed in the presence of EtOH 10–100 mM. Chronic EtOH treatment (12 days) did not shift sensitivity or maximum response to baclofen in CA1 neurons. These results suggest that $GABA_B$ receptors in this model were essentially insensitive to EtOH (Frye and Fincher 1996).

Melis et al. (2002) linked the long-lasting potentiation of GABAergic synapses on dopaminergic neurons in the VTA by systemic EtOH to an effect on presynaptic $GABA_B$ receptors. Moreover, the frequency (but not the amplitude) of mIPSCs was also significantly higher in VTA neurons of EtOH-treated animals compared to controls, further supporting an increased probability of presynaptic GABA release independent of neuronal discharge in VTA neurons treated with EtOH. Interestingly, the $GABA_B$ receptor antagonist, CGP 35348, shifted PPD to PPF in EtOH-treated animals by increasing the amplitude of the second evoked $GABA_A$ IPSC and without affecting $GABA_A$ IPSC in the saline-treated animals. In addition, both the frequency and the amplitude of mIPSCs were unaffected by CGP

35348 in both groups of mice. Thus, the PPD observed in the EtOH-treated mice could result from an increased probability of GABA release, which might in turn lead to activation of presynaptic $GABA_B$ receptors and decrease the second IPSC. These results further support the hypothesis that GABA levels are increased after EtOH exposure, leading to spillover onto presynaptic $GABA_B$ receptors, whose activation leads to inhibition of release (Hausser and Yung 1994; Melis et al. 2002).

In a recent study, Roberto et al. (2008) reported neuroadaptations in $GABA_B$ receptors in CeA after chronic EtOH exposure. The sensitivity of GABA IPSCs to the $GABA_B$ receptor antagonist CGP 55845A and agonist baclofen was decreased after chronic EtOH, suggesting downregulation of this system. Specifically, the $GABA_B$ receptor antagonist, CGP 55845A significantly increased the mean amplitude of evoked IPSCs (by 12 $\pm$ 5%) in CeA from naïve rats. This increase in the IPSC amplitude was associated with a significant decrease in PPF, suggesting a tonic activation of presynaptic $GABA_B$ receptors in naïve rats. In contrast, in CeA from EtOH-dependent rats, CGP 55845A did not alter the mean evoked IPSCs (98 $\pm$ 4%) and did not affect mean PPF. Baclofen (10 μM) markedly depressed evoked GABA-IPSC amplitudes in neurons of naïve rats (to 38% of control), with recovery during washout. The baclofen-induced inhibition of GABA IPSCs was significantly reduced (to 86% of control) in neurons of EtOH-dependent rats. In addition, in CeA neurons from EtOH-dependent rats, baclofen-induced depression was associated with a smaller increase of the PPF ratio of GABA IPSCs compared to that in neurons of naïve rats. These data suggest that the downregulation of the $GABA_B$ system associated with EtOH-dependence may explain in part the increased GABAergic tone reported in dependent rats (Roberto et al. 2008).

3 Neuropeptide Roles in Acute and Chronic Alcohol Actions

Neuropeptides are potent neuromodulators in the CNS whose actions are mediated via GPCRs. In contrast to classical neurotransmitters, neuropeptides are released in a frequency-dependent fashion and often have a longer half-life of activity after release. These factors, among others, enable neuropeptides to produce long-lasting effects on cellular functions such as excitatory and inhibitory synaptic transmission, neuronal excitability, and gene transcription (Gallagher et al. 2008). Thus, a long-lasting dysregulation of neuropeptides could have significant effects on the activity of neurons and consequently, behavior.

3.1 Corticotropin-Releasing Factor

Corticotropin-releasing factor (CRF) is a 41-amino acid polypeptide that has a major role in coordinating the stress response of the body by mediating hormonal,

autonomic, and behavioral responses to stressors. CRF (originally called corticotropin-releasing hormone, although the International Union of Pharmacology designation is CRF) was identified through classic techniques of peptide sequencing (Vale et al. 1981). Subsequently, genes encoding three paralogs of CRF—urocortins 1, 2, and 3 (*Ucn 1*, *Ucn 2*, *Ucn 3*), were identified by modern molecular biologic approaches. Ucn 2 and Ucn 3 are also referred to as stresscopin-related peptide and stresscopin, respectively. CRF and the urocortins have been implicated in the modulation of multiple neurobiologic systems, including those that regulate feeding, anxiety and depression, hypothalamic-pituitary-adrenal (HPA) axis signaling, and EtOH consumption (Hauger et al. 2006; Heilig and Koob 2007; Ryabinin and Weitemier 2006; Smith and Vale 2006). CRF and the Ucn peptides produce their effects by binding to the G protein-coupled CRF type 1 (CRF1R) and CRF type 2 (CRF2R) receptors. CRF binds to both receptors, but has greater affinity for the CRF1R (Bale and Vale 2004; Fekete and Zorrilla 2007; Hauger et al. 2006; Pioszak et al. 2008).

CRF1R and CRF2R are GPCRs that are predominantly positively linked to the activation of AC (Fig. 1), and recent reports also implicate other second messenger systems such as inositol triphosphate and PKC (Blank et al. 2003; Grammatopoulos et al. 2001). Using corticotrophins, Antoni et al. (2003) demonstrated a coupling of CRF1R to AC9 and AC7. The switch in coupling from AC9 to AC7 results in a more robust cAMP signal when CRF binds to the CRF1R (Antoni 2000; Antoni et al. 2003). It should be emphasized that AC7 is localized both postsynaptically (striatum, hippocampus) and presynaptically (nucleus accumbens, amygdala) (Mons et al. 1998a, b), and is anatomically positioned to receive signals from GPCRs on both dendrites and axon terminals.

Pharmacological and transgenic studies show that brain and pituitary CRF1Rs mediate many of the functional stress-like effects of the CRF system (Heinrichs and Koob 2004). CRF and the Ucn peptides have a wide distribution throughout the brain, but there are particularly high concentrations of cell bodies in the paraventricular nucleus of the hypothalamus, the basal forebrain (notably the extended amygdala), and the brainstem (Swanson et al. 1983). Ucn1 binds with equal affinity to CRF1R and CRF2R, and Ucn2 and Ucn3 are CRF2R agonists (Hauger et al. 2006; Pioszak et al. 2008). CRF and the Ucn peptides exert their behavioral and neuroendocrine actions through central hypothalamic and extrahypothalamic pathways (Hauger et al. 2006; Heilig and Koob 2007; Heinrichs and Koob 2004; Koob and Le Moal 2008).

Increasing evidence implicates CRF and its receptors in the synaptic effects of EtOH. EtOH induces release of CRF from the hypothalamus that initiates the activation of the HPA axis (Ogilvie et al. 1998). EtOH also modulates the extraneuroendocrine CRF system involved in behavioral stress responses, particularly in the amygdala. EtOH withdrawal induces an increase in CRF levels in the amygdala (Merlo Pich et al. 1995) and in the BNST (Olive et al. 2002).

The central administration of a CRF antagonist attenuates both EtOH self-administration and the anxiety-like response to stress observed during alcohol abstinence (Valdez et al. 2002) and administration of a CRFR antagonist into the

CeA reverses the anxiogenic-like effect of alcohol (Rassnick et al. 1993). Rats tested 3–5 weeks post alcohol withdrawal showed an anxiogenic-like response provoked by a mild restraint stress only in rats with a history of alcohol dependence. This stress-induced anxiogenic-like response was reversed by a competitive CRF1R antagonist (Valdez et al. 2003). The increased self-administration of alcohol observed during protracted abstinence also was blocked by a competitive CRF1R antagonist (Valdez et al. 2003). Gehlert et al. (2007) also described that a novel CRF1R antagonist, the 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine (MTIP) has advantageous properties for both clinical development and in preclinical alcoholism models. MTIP dose-dependently reversed anxiogenic effects of EtOH withdrawal, and blocked excessive alcohol self-administration in Wistar rats with a history of dependence (Gehlert et al. 2007). CRF also contributes to increased alcohol consumption in dependent animals, because increased EtOH self-administration is reduced by CRF1R antagonists in dependent animals but not in non-dependent animals (Funk et al. 2007; Overstreet et al. 2004) and by CRF1R deletion (Chu et al. 2007; Sillaber et al. 2002). More recently, it has been reported that chronic CRF1R antagonist treatment blocked withdrawal-induced increases in alcohol drinking by dependent rats, and tempered moderate increases in alcohol consumption (Roberto et al. 2010). These results have led to the hypothesis that negative emotional states (including anxiety-like states) contribute to the compulsive alcohol intake associated with dependence via negative reinforcement mechanisms (Koob 2008).

A recent review (Lowery and Thiele 2010) provides a comprehensive overview of preclinical evidence from rodent studies that suggest a promising role for CRFR antagonists in the treatment of alcohol abuse disorders. CRFR antagonists protect against excessive EtOH intake resulting from EtOH dependence without influencing EtOH intake in non-dependent animals. Similarly, CRFR antagonists block excessive binge-like EtOH drinking in non-dependent mice but do not alter EtOH intake in mice drinking moderate amounts of EtOH (Lowery and Thiele 2010). CRFR antagonists also protect against increased EtOH intake and relapse-like behaviors precipitated by exposure to a stressful event. Additionally, CRFR antagonists attenuate the negative emotional responses associated with EtOH withdrawal. The protective effects of CRFR antagonists are modulated by CRF1R. Finally, recent evidence has emerged suggesting that CRF2R agonists may also be useful for treating alcohol abuse disorders for review see (Lowery and Thiele 2010).

Low CRF concentrations can influence neuronal properties in the CNS (see (Aldenhoff et al. 1983; Siggins et al. 1985). CRF decreases the slow after hyperpolarizing potential in hippocampus (Aldenhoff et al. 1983) and CeA (Rainnie et al. 1992), and enhances R-type voltage-gated calcium channels in rat CeA neurons (Yu and Shinnick-Gallagher 1998). These and other data (Liu et al. 2004; Nie et al. 2004, 2009; Roberto et al. 2010; Ungless et al. 2003) also suggest that CRF plays an important role in regulating synaptic transmission in CNS. For example, in VTA dopamine neurons, CRF potentiates NMDA-mediated synaptic transmission via CRF_2 activation (Ungless et al. 2003), and we recently found that

CRF augments GABAergic inhibitory transmission in mouse CeA neurons via CRF1 activation (Fig. 1).

3.1.1 Corticotropin-Releasing Factor Actions in the Ventral Tegmental Area

The ventral tegmental area (VTA) receives CRF inputs from a number of sources including the limbic forebrain and the paraventricular nucleus of the hypothalamus (Rodaros et al. 2007). These CRF inputs form symmetric and asymmetric synapses, mostly onto dendrites that co-release either GABA or glutamate, respectively (Tagliaferro and Morales 2008). VTA dopamine neurons express both types of CRF receptors, CRF1R and CRF2R (Ungless et al. 2003), and approximately 25% of VTA dopamine neurons express the CRF binding protein (CRF-BP) (Wang et al. 2005; Wang and Morales 2008). CRF regulates dopamine neurons through a subtle interplay of effects at CRF1R, CRF2R, and CRF-BP. CRF increases the action potential firing rate in VTA dopamine neurons via CRF1R and involves a PKC-dependent enhancement of I_h (a hyperpolarization-activated inward current) (Wanat et al. 2008). CRF enhanced the amplitude and slowed the kinetics of IPSCs following activation of D2-dopamine and $GABA_B$ receptors. This action is postsynaptic and dependent on the CRF1R. The enhancement induced by CRF was attenuated by repeated in vivo exposures to psychostimulants or restraint stress (Beckstead et al. 2009).

CRF can induce a slowly developing, but transient, potentiation of NMDAR-mediated synaptic transmission (Ungless et al. 2003). This effect involves the CRF2R and activation of the protein kinase C pathway and the requirement of CRF-BP. However, the effect of CRF is restricted to a subset of dopamine neurons expressing large I_h currents (Ungless et al. 2003).

In addition to fast, excitatory glutamate-mediated synaptic transmission, dopamine neurons also express metabotropic glutamate receptors (mGluRs) which mediate slower, inhibitory synaptic transmission (Fiorillo and Williams 1998). The rapid rise and brief duration of synaptically released glutamate in the extracellular space mediates a rapid excitation through activation of ionotropic receptors, followed by inhibition through the mGluR1 receptor (Fiorillo and Williams 1998). CRF can enhance these mGluRs via a CRF2R-PKA pathway that stimulates release of calcium from intracellular stores (Riegel and Williams 2008). The CRF modulation of VTA synaptic activity is very complex because CRF has diverse actions on dopamine neurons that are excitatory and inhibitory. In summary, the excitatory effects of CRF on dopamine neurons appear to affect fast events (e.g. action potential firing rate and NMDAR-mediated synaptic transmission), whereas the inhibitory effects involve slow forms of synaptic transmission. Another important aspect is that CRF1R-mediated effects do not involve interactions with the CRF-BP, whereas CRF2R-mediated effects do.

It is speculated that these effects on short-term plasticity phenomena may modulate longer lasting forms of plasticity. For example, NMDAR activation is

required for the induction of long-term potentiation in VTA dopamine neurons (Bonci and Malenka 1999; Borgland et al. 2010).

3.1.2 Corticotropin-Releasing Factor Actions in the Central Amygdala

The central amygdala (CeA) contains CRF receptors and abundant CRF-containing fibers (De Souza et al. 1984) (Uryu et al. 1992); CRF itself is generally co-localized in CeA neurons together with GABA (Eliava et al. 2003) (Asan et al. 2005). Acute EtOH augments evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) by increasing GABA release in both mouse (Bajo et al. 2008; Nie et al. 2004) and rat CeA neurons (Roberto et al. 2003, 2004).

CRF1Rs mediate the EtOH-induced augmentation of IPSCs in mouse CeA (Nie et al. 2004; Nie et al. 2009) via the PKCε signaling pathway (Bajo et al. 2008; Nie et al. 2004). Both CRF and EtOH augment evoked IPSCs in mice CeA neurons, and CRF1R (but not CRFR2) antagonists blocked both CRF and EtOH effects. In addition, CRF and EtOH augment IPSCs in wild-type and CRF2R knockout mice, but not in CRF1R knockout mice (Nie et al. 2004).

New electrophysiological data showed that CRF, like EtOH, also enhances GABAergic transmission in the rat CeA (Roberto et al. 2010). As in mice, CRF and EtOH actions involve presynaptic CRF1R activation at the CeA GABAergic synapses. Interestingly, the interactions between the CRF and GABAergic systems in the CeA may play an important role in alcohol reward and dependence (Roberto et al. 2010). These results suggest that the presynaptic effect of EtOH on GABA release in rodent CeA involves CRF1R and perhaps release of CRF itself. Thus, superfusion of CRF has an effect on GABA IPSCs equivalent to that of EtOH: an increase in IPSC amplitude of about 30–50%. Furthermore, both CRF and EtOH decreased PPF of IPSCs in mouse and rat neurons, and the effects of both were selectively blocked by CRF1R antagonists. In addition, both EtOH and CRF increase the frequency of GABAR-mediated mIPSCs, and this effect is blocked by CRF1R antagonists (Nie et al. 2004; 2009; Roberto et al. 2010). Thus, EtOH probably enhances the release of GABA by activating CRF1R on GABAergic terminals (Nie et al. 2009; Roberto et al. 2010). Conversely, CRF1R antagonists directly increased PPF of IPSCs and decreased mIPSC frequencies, consistent with decreased GABA release, thus opposing EtOH effects. Because GABA and CRF are often co-localized in CeA neurons, the EtOH-elicited GABA release may involve release of the CRF peptide itself, perhaps even from the terminals synapsing on autoreceptors on the same cell bodies or on collaterals from other GABAergic interneurons. Thus, this example raises the possibility of involvement of other, secondary messengers in EtOH effects on GABAergic terminals.

Chronic EtOH exposure produces functional adaptation of the CRF system in CeA (Hansson et al. 2006, 2007; Sommer et al. 2008; Weiss et al. 2001). In the study by Roberto et al. electrophysiological experiments were performed 2–8 h after preparation of CeA slices from EtOH-dependent or naïve control rats. Interestingly, in CeA of dependent rats, the ability of maximal (200 nM) and a

submaximal (100 nM) concentrations of CRF to augment evoked IPSCs was significantly enhanced compared to naïve CeA. A greater effect of CRF1R antagonists on basal IPSCs of dependent rats was also reported. The greater effect of CRF and CRF1R antagonists may reflect increased tonic release of endogenous CRF, constitutive CRF1R activation, increased receptor number, and/or sensitization of CRF1R in CeA of dependent rats. These combined findings suggest an important EtOH–CRF interaction on GABAergic transmission in the CeA that markedly increases during development of EtOH dependence (Roberto et al. 2010).

CRF-related peptides serve as hormones and neuromodulators of the stress response and play a role in affective disorders. It has been shown that excitatory glutamatergic transmission is modulated by two endogenous CRF-related peptide ligands, CRF rat/human (r/h) and Ucn I, within the CeA and the lateral septum mediolateral nucleus (LSMLN) (Liu et al. 2004). Activation of these receptors exerts diametrically opposing actions on glutamatergic transmission in these nuclei. In the CeA, CRF(r/h) depressed excitatory glutamatergic transmission through a CRF1R-mediated postsynaptic action, whereas Ucn I facilitated synaptic responses through pre and postsynaptic CRF2R-mediated mechanisms. Conversely, in the lateral septum mediolateral nucleus (LSMLN), CRF induced a CRF1R-mediated facilitation of glutamatergic transmission via postsynaptic mechanisms, whereas Ucn I depressed EPSCs by postsynaptic and presynaptic CRF2R-mediated actions. Furthermore, antagonists of these receptors also affected glutamatergic neurotransmission, indicating a tonic endogenous modulation at these synapses (Liu et al. 2004). These data show that CRF receptors in CeA and LSMLN synapses exert and maintain a significant synaptic tone and thereby regulate excitatory glutamatergic transmission. The results also suggest that CRF receptors may provide novel targets in affective disorders and stress (Liu et al. 2004).

3.1.3 Corticotropin-Releasing Factor Actions in the Bed Nucleus of the Stria Terminalis

The bed nucleus of the stria terminalis (BNST), a brain region associated with anxiety, has enriched expression of CRF (Ju and Han 1989) and CRFRs (Van Pett et al. 2000). A component of the extended amygdala, the BNST is anatomically well-situated to integrate stress and reward-related processing in the CNS, regulating activation of the HPA axis and reward circuits. The BNST receives dense GABAergic and CRF input from the CeA (Sakanaka et al. 1986), suggesting that CRF regulation of function in the BNST is critical for shaping BNST output. Pharmacological studies suggest that CRF signaling in the BNST is involved in anxiety (Lee and Davis 1997) and stress-induced relapse to cocaine self-administration (Erb and Stewart 1999). Moreover, a stimulus that promotes anxiogenic responses, the withdrawal of rodents from chronic EtOH exposure, produces rises in extracellular levels of CRF in the BNST (Olive et al. 2002).

Interactions between CRF and GABAergic transmission in BNST have been reported to play a role in regulating stress and anxiety (Kash and Winder 2006). In this study the actions of CRF on GABAergic transmission in the ventrolateral region of the BNST (vlBNST) were examined. This region projects to both the VTA (Georges and Aston-Jones 2002) and the PVN of the hypothalamus (Cullinan et al. 1993), thus providing a point of access to both reward and stress pathways. Using whole-cell recordings in a BNST slice preparation, Kash et al. found that CRF enhances GABAergic transmission. Their pharmacological and genetic experiments suggest that CRF and urocortin CRF enhance postsynaptic responses to GABA through activation of the CRF1R.

In the same laboratory, a recent study showed the action of dopamine on cellular and synaptic function in the BNST. Kash et al. (2008) directly assessed the ability of dopamine to modulate neuronal function in the BNST using an ex vivo slice preparation. These investigators demonstrated a rapid and robust dopamine-induced enhancement of excitatory transmission in the BNST. This enhancement is activity-dependent and requires the downstream action of CRF1R, suggesting that dopamine induces CRF release through a local network mechanism. Furthermore, it was found that both in vivo and ex vivo cocaine induced a dopamine receptor and CRF1R-dependent enhancement of a form of NMDA receptor-dependent short-term potentiation in the BNST. These data highlight a direct and rapid interaction between dopamine and CRF systems that regulate excitatory transmission and plasticity in a brain region key to reinforcement and reinstatement. Because a rise in extracellular dopamine levels in the BNST is a shared consequence of multiple classes of drugs of abuse, this suggests that the CRF1R-dependent enhancement of glutamatergic transmission in this region may be a common key action of substances of abuse (Kash et al. 2008).

Francesconi et al. (2009a, b) investigated the effects of protracted withdrawal from alcohol in the juxtacapsular nucleus of the anterior division of the BNST (jcBNST). The jcBNST receives robust glutamatergic projections from the Basolateral amygdal (BLA), the postpiriform transition area, and the insular cortex as well as dopamine inputs from the midbrain. In turn, the jcBNST sends GABAergic projections to the medial division of the central nucleus of the amygdala (CeAm) as well as other brain regions. These investigators described a form of long-term potentiation of the intrinsic excitability (LTP-IE) of neurons of the jcBNST in response to high-frequency stimulation (HFS) of the stria terminalis that was impaired during protracted withdrawal from alcohol (Francesconi et al. 2009b). Administration of the selective CRF1R antagonist (R121919), but not of the CRF2R antagonist (astressin 2B), normalized jcBNST LTP-IE in animals with a history of alcohol dependence (Francesconi et al. 2009b). In addition, repeated, but not acute, administration of CRF itself produced a decreased jcBNST LTP-IE. These investigators also showed that dopaminergic neurotransmission is required for the induction of LTP-IE of jcBNST neurons through dopamine D1 receptors (Francesconi et al. 2009b). Thus, activation of the central CRF stress system and altered dopaminergic neurotransmission during protracted withdrawal from alcohol and drugs of abuse may contribute to the disruption of LTP-IE in the jcBNST.

Impairment of this form of intrinsic neuronal plasticity in the jcBNST could result in inadequate neuronal integration and reduced inhibition of the CeA, contributing to the negative affective state that characterizes protracted abstinence in postdependent individuals (Francesconi et al. 2009a, b).

3.1.4 Corticotropin-Releasing Factor Actions in the Basolateral Amygdala

Liu et al. (2004) demonstrated that CRF and its related family of peptides act differentially at CRF1 versus CRF2 synaptic receptors to facilitate or depress excitatory transmission in CeA and lateral septum mediolateral nucleus. Notably, the effects of CRF and its ligands occurred without any apparent direct action on membrane potential or membrane excitability, suggesting that the role of CRF at these limbic synapses is that of a 'neuroregulator'. The investigators suggested pre and postsynaptic loci for CRF1 and CRF2 receptors within the glutamatergic CeA and LSMN synapses. Although both synapses exhibit a comparable pre and postsynaptic location of CRF1 and CRF2 receptors, their functions (facilitation versus depression of glutamatergic transmission) are opposite within each synapse (Gallagher et al. 2008). Liu et al. (2004) also demonstrated that endogenous CRF ligands induce a tonic effect on excitatory glutamatergic transmission at synapses within both of these nuclei since application of competitive, selective CRF1 or CRF2 receptor antagonists resulted in an enhancement or depression of glutamatergic EPCS. A similar tonic endogenous action of CRF ligands was not observed under control conditions in the medial prefrontal cortex (Orozco-Cabal et al. 2006). This latter result further emphasizes that CRF effects are different depending upon the CNS synapse being investigated. Most of these studies in the Gallagher group aimed to investigate the action of CRF on glutamatergic synapses in relation to cocaine administration. There is very poor data on EtOH–CRF–glutamate interaction.

Taken together these data suggest that a dysregulation of the extrahypothalamic CRF function is a major determinant of vulnerability to high alcohol intake and maintenance of alcohol and drug dependence.

3.1.5 Neuropeptide Y

Neuropeptide Y (NPY) is an inhibitory peptide produced in abundance in the hypothalamus, and phylogenetically conserved across species (Allen et al. 1986). NPY is involved in regulation of food and water intake. It has recently been ascribed its prominent role in the aversive aspects of alcohol withdrawal and relapse via their actions in the CeA. Endogenous NPY reduces anxiety via actions in the amygdala (Heilig et al. 1993; Sajdyk et al. 2002) and suppresses alcohol drinking in rats (Gilpin et al. 2003) via its actions in CeA (Gilpin et al. 2008a, b; Thorsell 2008). More specifically, NPY microinjection into the CeA exhibits an enhanced ability to suppress alcohol drinking in certain subpopulations of drinkers, including rats that are made dependent on alcohol via vapor inhalation.

NPY is generally co-localized with GABA in inhibitory interneurons. NPY mediates its actions by interacting with a family of G protein-coupled receptors (GPCRs), at least five of which have been cloned and designated Y1, Y2, Y4, Y5, and Y6. These receptors are widely distributed throughout the brain. NPY has also been shown to be a regulator of neuronal excitability in hippocampus, where its cellular actions have been most extensively studied (Colmers et al. 1991). In the amygdala, NPY has anxiolytic effects that are mediated via activation of Y1 receptors (Heilig et al. 1993). NPY neurons in the amygdala project to the BNST (Allen et al. 1984), which also contains Y1 receptors and Y1 and Y2 receptor mRNA. Further, the CeA receives NPYergic input from the nucleus of the solitary tract, arcuate nucleus, and the lateral septum (see (Kask et al. 2002) for a review). Y1, Y2, and Y5 receptors, and receptor mRNA are found in the amygdala, and each of these receptor subtypes has been implicated in anxiety (Kask et al. 2002). Y2 receptors are thought to act presynaptically as auto-receptors providing negative feedback to NPYergic nerve terminals, whereas Y1 receptors appear to act postsynaptically (Kask et al. 2002; Wolak et al. 2003).

Many in vivo studies point to the involvement of NPY in mediating some of the behavioral effects of EtOH (Caberlotto et al. 2001; Cippitelli et al. 2010; Rimondini et al. 2005). NPY KO mice show increased EtOH preference but blunted behavioral responses to EtOH, while NPY overexpressors show a lower preference and increased sensitivity to EtOH (Thiele et al. 1998). Likewise, increased NPY expression in the CeA was noted in two independent strains of alcohol-preferring rats (Hwang et al. 1999). There were increased levels of NPY in the paraventricular nucleus of the hypothalamus (PVN) and arcuate nucleus of EtOH-preferring rats and decreased NPY levels in the CeA of EtOH-preferring rats, suggesting an inverse relationship between NPY levels in the CeA and EtOH consumption. Additionally, alcohol-preferring rats show significant decreases in both cAMP-responsive element-binding protein (CREB) and NPY levels in the CeA and medial amygdala, but not the basolateral amygdala (Pandey et al. 2005). Further, virally mediated alterations in NPY levels in the CeA differentially affect EtOH consumption in rats with low and high basal levels of anxiety (Primeaux et al. 2006). Also, recent genetic and pharmacological evidence indicates that C57BL/6 J mice have low NPY levels in CeA compared to DBA/2 mice, suggesting that NPY contributes to the high EtOH consumption characteristic of C57BL/6 J mice (Hayes et al. 2005).

Electrophysiologic findings suggest that NPY and EtOH have a similar profile of actions (Ehlers et al. 1998a, b, 1999). Increased sensitivity to NPY and CRF was observed in cortex and amygdala after chronic EtOH exposure, as measured by EEG activity and event-related potentials (Slawecki et al. 1999). Modulation of amygdala EEGs by NPY differs in naïve P and NP rats, suggesting that NPY has different neuromodulatory effects in these two strains (Ehlers et al. 1998a). Furthermore, NPY antagonizes the effects of CRF in the amygdala. However, to date neither the cellular actions of NPY in neither the CeA nor its interactions with EtOH or CRF, have been fully characterized. Recent findings by Gilpin et al. (2009, 2011) show that NPY superfusion decreased baseline GABAergic

transmission in CeA slices and blocked the alcohol-induced enhancement of inhibitory transmission in CeA via presynaptic Y2 receptors.

Recently, it has been shown that NPY and CRF have opposing effects on stress and anxiety as well as on synaptic activity in BNST (Heilig et al. 1994; Kash and Winder 2006). Kash and Winder found that NPY and CRF inhibit and enhance GABAergic transmission, respectively: NPY depresses GABAergic transmission through activation of the Y2 receptors, whereas CRF and urocortin enhance GABAergic transmission through activation of CRF1 receptors. Further, NPY appears to reduce GABA release, whereas CRF enhances postsynaptic responses to GABA, suggesting potential anatomic and cellular substrates for the robust behavioral interactions between NPY and CRF in the extended amygdala.

3.1.6 Orphanin FQ/nociceptin (OFQ/N)

Nociceptin (known also as orphanin FQ) is the most recently discovered member of the endogenous opioid peptide family, albeit nearly 15 years ago. Nociceptin mediates or influences many behavioral, psychological, and neurobiological processes, including memory, anxiety, stress, and reward (Economidou et al. 2008; Martin-Fardon et al. 2010; Murphy 2010). The hepta decapeptide nociceptin is the endogenous ligand of the nociceptin opioid receptor (NOR), previously referred to as opiate receptor-like1 (ORL1). NOR is a GPCR that belongs to the opioid receptor family (Mogil et al. 1996; Mogil and Pasternak 2001). In rodents, moderate to high levels of NOR mRNA are detected in cerebral cortex, nucleus accumbens, amygdala, dorsal raphe nucleus, and hippocampus (Harrison and Grandy 2000). Nociceptin has a high structural homology with opioid peptides, especially dynorphin A (Meunier et al. 1995; Reinscheid et al. 1995), but nociceptin does not bind to MOR, DOR or KOR (μ, δ and κ-opioid receptors) and opioid peptides do not bind NOR (Lachowicz et al. 1995; Reinscheid et al. 1995). Nociceptin inhibits forskolin-stimulated cAMP formation (see Harrison and Grandy 2000; Hawes et al. 2000), and protein kinase C (PKC), MAP kinases and phospholipase A2 have been linked to NOR (Fukuda et al. 1998; Hawes et al. 2000; Lou et al. 1998).

At the cellular level, nociceptin acts at NOR to augment K^+ conductances in amygdalar (Meis and Pape 1998, 2001), hippocampal (Amano et al. 2000; Ikeda et al. 1997; Madamba et al. 1999; Tallent et al. 2001; Yu and Xie 1998) and thalamic neurons (Meis 2003; Meis et al. 2002), thus depressing cell excitability. Nociceptin has also been shown to decrease Ca^{2+} currents (Abdulla and Smith 1997; Calo et al. 2000; Connor et al. 1999; Henderson and McKnight 1997; Larsson et al. 2000) and reduce the amplitude of both non-NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) and IPSCs in rat lateral amygdala (Meis et al. 2002).

Roberto and Siggins (2006) found that nociceptin did not significantly alter the resting membrane potential, input resistance, or spike amplitude, in accord with the results reported by others in CeA (Meis and Pape 1998) and for other brain regions (Ikeda et al. 1997; Madamba et al. 1999; Tallent et al. 2001). However, nociceptin

dose-dependently reduced $GABA_A$ IPSCs. This inhibition of GABAergic transmission was reversible on washout (Roberto and Siggins 2006). Nociceptin also concomitantly increased the PPF of IPSCs, and decreased the frequency of mIPSCs, suggesting decreased GABA release. Thus, nociceptin decreases GABAergic transmission by reducing GABA release at CeA synapses (Roberto and Siggins 2006). Interestingly, nociceptin applied before EtOH completely prevented the EtOH-induced enhancement of GABAergic transmission in CeA. On the other hand, EtOH alone significantly increased both the evoked IPSCs and mIPSC frequencies, and decreased the PPF ratio; nociceptin in the presence of EtOH completely reversed these EtOH effects opposing the EtOH increase of GABA release (Roberto and Siggins 2006). These investigators also found that the nociceptin-induced decrease of GABAergic transmission was larger in EtOH-dependent rats and might reflect neuroadaptations associated with EtOH dependence.

The functional interactions of neuropeptides (CRF, NPY, nociceptin) with GABAergic and glutamatergic systems may play major roles in the acute effects of EtOH on GABAergic and glutamatergic transmission. Understanding the underlying mechanisms of these interactions may offer a possible avenue for restoring "normal" function following chronic drug exposure. The neuroadaptations induced by chronic EtOH on GABAergic and glutamatergic systems may represent homeostatic or compensatory mechanisms in response to the acute EtOH actions on these systems.

4 Conclusions

In this review we have focused on acute and chronic EtOH actions on synaptic transmission. It is not possible to cover all aspects of this topic, and thus we have focused on describing the best established EtOH actions. As the review attests, EtOH affects numerous aspects of synaptic transmission both directly and indirectly, to alter brain function and behavior. Acute exposure to EtOH generally increases the function of cys-loop ligand-gated ion channels, with prominent effects of $GABA_A$ and glycine receptors. These actions increase synaptic and extra-synaptic inhibition and are thought to contribute to sedation and other aspects of intoxication. Ionotropic glutamate and P2X receptors are generally inhibited by acute EtOH exposure, with some noted exceptions. The inhibitory effect on ionotropic glutamate receptors is most prominent at NMDARs and on NMDAR-mediated synaptic responses, and this inhibitory action is thought to contribute to cognitive impairment produced by EtOH. At present, the postsynaptic EtOH effects on neurotransmitter receptors appear to occur within the receptor molecules themselves, although more work is needed to elucidate the roles of post-translation modification. On the presynaptic side, acute EtOH generally potentiates GABA release, contributing to the enhanced neuronal inhibition produced by the drug. The molecular mechanisms involved in EtOH potentiation of GABA release remain to be fully explored. EtOH also alters other aspects of synaptic transmission involving amino acid transmitters and monoamines.

The net result of the EtOH effects of transmission seems to be to dampen synaptic excitation in many brain regions and reduce most forms of synaptic plasticity (with noted exceptions).

Chronic exposure to EtOH, whether by forced administration or ingestion, generally enhances the function of NMDARs, most often those containing the NR2B subunit. Increases in glutamate release and responses to some other glutamate receptors are also observed following chronic exposure. The net effect of these increases in glutamatergic transmission appears to be a hyperexcitable CNS state during withdrawal that contributes to withdrawal symptoms and relapse. Excitotoxicity might be another result of this hyper-glutamatergic state. In general, acute EtOH effects on glutamate receptor function and glutamatergic transmission are intact even after subchronic or chronic EtOH exposure, suggesting that behavioral tolerance is not a simple function of loss of pharmacological effects at these synapses. At GABAergic synapses, chronic EtOH generally alters either the efficacy of inhibitory synaptic transmission or the types of receptors involved in transmission. Extrasynaptic $GABA_A$ receptor-mediated synaptic responses are also altered, leading to changes in tonic current in the postsynaptic neuron. The pattern of chronic EtOH effects on GABAergic transmission varies considerably across brain regions, making this subject a rich and important area for future investigation. The resultant alterations in patterns of GABAergic transmission in key brain regions may contribute to EtOH tolerance, dependence and drug intake. More work is needed to determine the exact pattern of changes in GABAergic inhibition across brain regions, and how these changes contribute to aspects of alcohol use disorders including tolerance, dependence, and escalating intake.

The modulatory effects of neuropeptides have become subjects of intense investigation in the alcohol research field. Neuropeptides implicated in stress responses, such as CRF, appear to contribute to stress–EtOH interactions as well as drinking and relapse. Acute EtOH exposure alters the release of some neuropeptides, while others alter synaptic transmission in ways that interfere with the actions of EtOH. Chronic EtOH exposure also appears to alter neuropeptide modulatory actions. In addition to providing tools for investigation of mechanisms involved in EtOH actions, the neuropeptides may also provide new avenues for pharmacotherapies that could be used in the treatment of alcohol use disorders. Researchers have just begun to explore the alcohol-related actions of a few of the many neuropeptides found in the brain. Thus, more work remains to fully define how peptides participate in the neural actions of alcohol.

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Signaling Pathways Mediating Alcohol Effects

Dorit Ron and Robert O. Messing

Abstract Ethanol's effects on intracellular signaling pathways contribute to acute effects of ethanol as well as to neuroadaptive responses to repeated ethanol exposure. In this chapter we review recent discoveries that demonstrate how ethanol alters signaling pathways involving several receptor tyrosine kinases and intracellular tyrosine and serine-threonine kinases, with consequences for regulation of cell surface receptor function, gene expression, protein translation, neuronal excitability and animal behavior. We also describe recent work that demonstrates a key role for ethanol in regulating the function of scaffolding proteins that organize signaling complexes into functional units. Finally, we review recent exciting studies demonstrating ethanol modulation of DNA and histone modification and the expression of microRNAs, indicating epigenetic mechanisms by which ethanol regulates neuronal gene expression and addictive behaviors.

Keywords Phosphorylation · Signal transduction · Growth factor · Epigenetic · Kinase

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D. Ron · R. O. Messing (✉)
Ernest Gallo Clinic and Research Center, University of California San Francisco,
5858 Horton Street, Suite 200, Emeryville, CA 94608, USA
e-mail: romes@gallo.ucsf.edu

D. Ron
e-mail: dron@gallo.ucsf.edu

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Contents

1 Introduction ... 88
2 Serine–Threonine Kinases ... 88
2.1 cAMP-Dependent Protein Kinase A (PKA) ... 89
2.2 Protein Kinase C (PKC) ... 93
2.3 Extracellular Signal-Regulated Kinases (ERKs) ... 97
2.4 P13K, AKT and GSK3beta ... 99
2.5 mTOR ... 100
3 Tyrosine Kinases ... 101
3.1 Receptor Tyrsosine Kinases ... 101
3.2 The Src Family of Protein Tyrosine Kinases ... 104
4 Scaffolding Proteins ... 106
4.1 RACK1 ... 106
4.2 PSD-95 ... 107
4.3 Homer ... 108
5 Epigenetic Regulation of Gene Expression ... 108
5.1 DNA Methylation ... 109
5.2 Histone Acetylation and Up-Regulation of Neuropeptide Y ... 110
5.3 MicroRNA ... 111
6 Summary ... 111
References ... 113

1 Introduction

Ethanol is a psychoactive substance with rewarding and sedative-hypnotic properties that stem largely from its acute effects on specific signaling proteins that lead to changes in localization and post-translational modifications, gene expression and neuronal excitability. Neurons adapt to repeated ethanol exposure through homeostatic changes in cellular signaling pathways that serve to maintain nervous system function in the presence of ethanol. Such neuroadaptations are thought to contribute to addiction partly because the absence of ethanol produces an aversive withdrawal state that negatively reinforces continued ethanol consumption. Such neuroadaptive changes include long-term changes in gene expression. This chapter reviews recent advances in the field of signal transduction in *Drosophila melanogaster* and rodents as it relates to alcohol use disorders, with emphasis on mechanisms that hold promise for discovery of new drug targets for treatment. Earlier findings are described in (Ron and Jurd 2005; Newton and Ron 2007; Nagy 2008; Lee and Messing 2008).

2 Serine–Threonine Kinases

Serine/threonine kinases are a large and heterogeneous group of enzymes that phosphorylate protein substrates on serine or threonine residues. Some are receptors (e.g. TGFβ receptors) but the majority are intracellular such as protein

kinases A, B (also known as AKT), C, calcium/calmodulin-dependent protein kinases and mitogen-activated protein kinases.

2.1 cAMP-Dependent Protein Kinase A (PKA)

PKA plays a key role in learning and memory (Abel and Nguyen 2008) and in behavioral responses to drugs of abuse (Lee and Messing 2008). It exists as an inactive tetramer of two regulatory subunits and two catalytic subunits. Adenylyl cyclase (AC) activation catalyzes the hydrolysis of ATP to cyclic adenosine 3′, 5′-monophosphate (cAMP). cAMP activates PKA by binding to the regulatory subunits, causing their dissociation from catalytic subunits, which then become active (Brandon et al. 1997). All PKA subunits (RIα, RIβ, RIIα, RIIβ, Cα and Cβ) are expressed in distinct but overlapping patterns in the brain (Cadd and McKnight 1989). There are nine AC isoforms and all are regulated by subunits of heterotrimeric G-proteins (Cooper 2003). $G_s\alpha$ activates all except possibly AC8 (Wang and Storm 2003), while $G_{olf}\alpha$ activates AC5, and $G\beta\gamma$ activates AC2, AC4 and AC7. Conversely, $G_{i/o}\alpha$ inhibits AC1, AC5, AC6 and AC8, while $G\beta\gamma$ inhibits AC1. Production of cAMP can also be regulated by protein kinase C (PKC) which inhibits AC6 and activates AC2, AC4 and AC7, and by calcium which inhibits AC5 and AC6, activates AC8 and together with $G_s\alpha$ synergistically activates AC1 (Wang and Storm 2003; Cooper 2003).

2.1.1 Ethanol Regulation of AC/PKA Signaling

Like other addictive drugs, ethanol acutely increases levels of extracellular dopamine in the nucleus accumbens (NAc) (Di Chiara and Imperato 1988), which activates D1 dopamine receptors coupled to G_s and G_{olf}, and leads to activation of AC and PKA. Dopamine also activates D2 receptors coupled to $G_{i/o}$, which inhibits several AC isoforms. Dopamine activation of D2 receptors also releases $G\beta\gamma$ subunits, which stimulate G-protein-regulated inwardly rectifying K^+ (GIRK) channels, and inhibit L−, N−, and P/Q-type calcium channels. The net effect of these actions on ion channel function is to depress neuronal excitability. However, in NAc neurons, $G\beta\gamma$ activation of AC is required for dopamine-stimulated firing, which requires co-activation of D1 and D2 receptors (Hopf et al. 2003).

An important downstream regulator of dopaminergic signaling in striatal neurons is the dopamine- and cAMP-regulated neuronal phosphoprotein of 32 kDa (DARPP-32), which acts as a bidirectional switch that is regulated by phosphorylation (Svenningsson et al. 2005). PKA phosphorylation of Thr-34 makes DARPP-32 a potent inhibitor of the protein phosphatase PP1, which in turn amplifies PKA-mediated phosphorylation of substrates. Cyclin-dependent protein kinase 5 (Cdk5) phosphorylates DARPP-32 at Thr-75, which turns DARPP-32 into an inhibitor of PKA and antagonizes several acute effects of dopamine in the

striatum. DARPP-32 appears critical for ethanol reinforcement and reward since mice lacking DARRP-32 show reduced ethanol self-administration and conditioned place preference (Maldve et al. 2002; Risinger et al. 2001).

Ethanol activates AC/PKA/DARPP-32 signaling through several mechanisms. Ethanol increases levels of extracellular dopamine in the NAc (Di Chiara and Imperato 1988; Weiss et al. 1993) by increasing firing of ventral tegmental area (VTA) dopamine neurons (Gessa et al. 1985; Brodie et al. 1990). Ethanol also enhances dopamine D1 receptor-mediated activation of AC (Rex et al. 2008). In addition, ethanol indirectly activates G_{olf}-coupled adenosine A2a receptors by inhibiting adenosine reuptake through type I equilibrative nucleoside transporters, thereby increasing extracellular concentrations of adenosine (Nagy et al. 1990; Choi et al. 2004). Low doses of ethanol and other addictive drugs such as opiates, cannabinoids and nicotine can act synergistically to stimulate ACs through combined effects at A2a receptors, which activate G_{olf}, and dopamine D2 receptors, which cause release of $G\beta\gamma$ subunits (Yao et al. 2003; Yao et al. 2002). These events result in cAMP response element (CRE)-mediated gene expression not only in the NAc but also in several other limbic brain regions (Asyyed et al. 2006).

An important substrate of PKA is the cyclic AMP response element binding protein (CREB), a transcription factor activated by phosphorylation at Ser-133 by PKA and also by calcium/calmodulin-dependent protein kinase IV, or mitogen- and stress-activated protein kinases (MSK1 and 2) (Lonze and Ginty 2002; Hauge and Frodin 2006). In rats, chronic consumption of ethanol for several weeks decreases Ser-133 phosphorylated CREB (p-CREB) in the striatum (Li et al. 2003) and diminishes the ability of an acute ethanol challenge to increase p-CREB and CREB function in the striatum and cerebellum (Yang et al. 1998a, b). During acute ethanol withdrawal, p-CREB is also decreased in several regions of the cerebral cortex (Pandey et al. 2001). These decreases in p-CREB may relate to increased expression of protein kinase inhibitor α (PKIα), as demonstrated in the prefrontal cortex (PFC), NAc and amygdala by transcriptional profiling of brain tissue from Wistar rats subjected to chronic intermittent exposure for 2 weeks (Repunte-Canonigo et al. 2007). PKIα is a protein that acts as a pseudosubstrate inhibitor of PKA catalytic subunits. Since acute exposure to ethanol activates PKA signaling, up-regulation of PKIα can be considered a homeostatic compensatory response that normalizes PKA signaling during chronic ethanol exposure.

2.1.2 AC/PKA Signaling in Behavioral Responses to Ethanol

Several studies indicate a role for PKA in the intoxicating effects of ethanol. Inhibition of PKA through intracerebroventricular (ICV) administration of the selective PKA inhibitor KT5720 reduces the acute ataxic and hypnotic effects of ethanol in rats (Lai et al. 2007). Likewise, RIIβ knockout mice, which have reduced cAMP-stimulated PKA activity, show decreased sensitivity to hypnotic effects of ethanol (Thiele et al. 2000). In addition, pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice, which are predicted to have a

deficit in PKA signaling (Tanaka et al. 2004), show reduced hypothermic and hypnotic responses to ethanol. These studies suggest that ethanol-induced activation of PKA contributes to acute ataxic, hypothermic and sedative-hypnotic effects of ethanol.

In addition to regulating acute behavioral responses to ethanol, PKA signaling also regulates ethanol consumption. Thus, RIIβ knockout mice show reduced cAMP-stimulated PKA activity and increased ethanol intake (Thiele et al. 2000). Mice haplodeficient for the α and Δ isoforms of CREB show increased ethanol consumption (Pandey et al. 2004), while administration of the PKA inhibitor Rp-cAMPS into the central amygdala (CeA) (Pandey et al. 2003) or NAc shell (Misra and Pandey 2006) of Sprague–Dawley rats increases ethanol consumption and preference. These studies suggest that inhibiting PKA signaling, especially in the CeA and the NAc shell, leads to increased ethanol drinking through a CREB-dependent mechanism. In addition, in rats, long-term alcohol consumption with repeated episodes of deprivation produces a strong down-regulation of PACAP gene expression in the striatum that is reversed by treatment with glycine transport inhibitors, which also reduce relapse drinking in this model (Vengeliene et al. 2010), indicating that PACAP, which lies upstream of PKA, is part of a glycine-regulated gene network that undergoes neuroadaptation during long-term ethanol self-administration to promote relapse.

Not all studies, however, agree with these findings, but instead report that inhibition of PKA signaling increases acute effects of ethanol and decreases ethanol consumption. For example, mice that are haplodeficient for $G\alpha_s$, that express a dominant negative form of PKA (Wand et al. 2001), or lack the calcium-sensitive adenylyl cyclases AC1 and AC8 (Maas et al. 2005), show a more prolonged ethanol-induced loss of righting and less ethanol consumption than wild type mice. Systemic injection of the A2a receptor antagonist 3,7-dimethylpropargylxanthine, which is expected to reduce PKA signaling throughout the striatum, reduces ethanol consumption in Long-Evans (Arolfo et al. 2004) and Wistar rats (Thorsell et al. 2007). In addition, intra-striatal administration of a peptide that inhibits $G\beta\gamma$ and prevents both ethanol-stimulated nuclear translocation of PKA and PKA-stimulated gene expression, decreases ethanol intake in Long-Evans rats (Yao et al. 2002).

The differences between these studies and those that find reduced sensitivity to ethanol and increased ethanol drinking upon inhibition of AC/PKA signaling may relate to differential duration or level of exposure to ethanol, to effects of global versus local pharmacological and genetic manipulations, compensatory effects of gene targeting that alter non-PKA pathways, or, perhaps, effects of genetic background in the different mouse and rat strains used in these studies. However, experiments in selected and inbred lines of rats and mice do support a role for decreased PKA signaling in the amygdala and NAc shell in promoting ethanol drinking. For example, levels of CREB and p-CREB are lower in the NAc shell of high ethanol preferring C57BL/6 mice compared with DBA/2 mice, which are a low ethanol preferring strain. In addition, alcohol-preferring (P) rats show lower

levels of pCREB and CREB DNA binding activity in the CeA and medial amygdala (MeA) than alcohol non-preferring (NP) rats (Pandey et al. 1999a).

2.1.3 PKA and CREB Regulation of Anxiety and Ethanol Consumption

Studies by Pandey and colleagues have identified key roles for amygdala PKA, CREB and the CREB-regulated gene neuropeptide Y (NPY) in the co-regulation of anxiety and ethanol drinking. NPY is abundantly expressed in the brain and is anxiolytic when administered into the central nervous system (CNS) (Heilig 2004). Knockout of the NPY gene in mice increases ethanol consumption, while transgenic overexpression of NPY reduces it (Thiele et al. 1998). P rats drink excessively and have lower levels of p-CREB and NPY in the amygdala compared with NP rats; P rats also show greater anxiety-like behavior than NP rats (Pandey et al. 2005). Self-administration of increasing concentrations of ethanol (7–12% over 10 days) in a two-bottle choice paradigm, or injection of 1 g/kg ethanol, normalizes anxiety-like behavior in P rats. These findings are associated with increased p-CREB and expression of NPY in the CeA and MeA of ethanol-treated P rats (Pandey et al. 2005). Infusion of the PKA activator Sp-cAMP into the CeA of P rats increases local p-CREB and NPY levels, decreases ethanol self-administration and normalizes their heightened anxiety-like behavior (Pandey et al. 2005). Conversely, in NP rats, infusion of the PKA inhibitor Rp-cAMP into the CeA decreases local p-CREB and NPY, increases anxiety-like behavior and increases ethanol intake (Pandey et al. 2005). The importance of NPY in these behavioral changes has been demonstrated by infusing NPY into the amygdala of P rats, which mimics the effect of Sp-cAMPs by decreasing anxiety-like behavior and ethanol intake.

Increased anxiety that accompanies alcohol withdrawal is argued to be one of the negatively reinforcing factors that promotes ethanol consumption (Koob 2009). Support for this concept stems from studies of diminished amygdala PKA signaling and NPY expression that accompany ethanol withdrawal in rats. One day after withdrawal from chronic daily intake of ethanol, Sprague–Dawley rats show increased anxiety-like behavior (Pandey et al. 2003), which is associated with decreased p-CREB (Pandey et al. 2003) and NPY (Roy and Pandey 2002) in the CeA and MeA. Sp-cAMPS infused into the CeA normalizes CREB phosphorylation and NPY expression, and prevents withdrawal-induced anxiety in these rats (Pandey et al. 2003; Zhang and Pandey 2003). In ethanol na rats, infusion of the PKA inhibitor Rp-cAMPS into the CeA decreases local p-CREB and NPY and increases both anxiety and ethanol consumption (Pandey et al. 2003; Zhang and Pandey 2003). Furthermore, infusion of NPY into the CeA prevents Rp-cAMPS-induced decreases in ethanol preference (Pandey et al. 2003). Intra-amygdalar infusion of NPY also reduces ethanol intake in P rats after multiple episodes of alcohol deprivation (Gilpin et al. 2003) and reduces withdrawal-induced increases in ethanol consumption in Wistar rats (Gilpin et al. 2008). These results indicate

that deficient PKA and NPY signaling in the amygdala are critical for increased anxiety and drinking that accompany alcohol withdrawal.

2.2 Protein Kinase C (PKC)

The PKC family of serine–threonine kinases mediates signals derived from lipid second messengers. The members of this family share similar catalytic domains but can be subdivided into four classes based on differences in their regulatory domains that alter structure and function (Rosse et al. 2010; Newton 2010). The classical or conventional cPKCs (α, β, γ) are activated by diacylcglycerol (DAG) and calcium. Novel nPKCs (δ, ε, η, θ) are activated by diacylglycerol but not by calcium. Atypical PKCs (ι or λ in mice, and ζ), do not require calcium or diacylglycerol for activation, but can be activated by phosphatidylinositols, phosphatidic acid, arachidonic acid and ceramide, and by interaction with the partitioning defective 6 (PAR6)-CDC42 complex (Hirai and Chida 2003; Rosse et al. 2010). Recently, a fourth group of PKC-related kinases (PKN1, PKN2, PKN3) has been included as a PKC subfamily; they are activated by the small G-proteins Rac and Rho (Rosse et al. 2010).

DAG, generated by activation of phospholipase C (PLC), is the most studied lipid activator of PKC signaling (Fukami et al. 2010). Among the PLC isoforms, activation of β and γ subtypes has been best described. PLCβ is activated by G$\beta\gamma$ or Gα_q subunits of heterotrimeric G-proteins released upon ligand binding to G-protein coupled receptors. Activation of receptor tyrosine kinases leads instead to recruitment, tyrosine phosphorylation, and activation of PLCγ. DAG can also be generated as a result of receptor-mediated activation of phospholipase D (Nishizuka 1995). PKC activation is generally associated with translocation of PKC from one cellular compartment to another containing lipid activators and proteins that bind the activated kinase near substrates. Here we summarize recent work on ethanol and PKC, focusing on three PKC isozymes, PKCε, PKCγ and PKCδ (see also section on RACK1).

2.2.1 Ethanol Regulation of PKC Activity

Ethanol has been reported to activate, inhibit or have no effect on PKC activity in vitro, depending on experimental conditions (reviewed in (Stubbs and Slater 1999). Recently, ethanol was reported to bind to PKCε and inhibit PKCε activity when assayed in vitro in the presence of DAG plus phosphatidylcholine and phosphatidylserine (Das et al. 2009). However, using DAG and phosphatidylserine with Triton-X-100 micelles, we previously found that ethanol does not alter the activity of PKC in vitro (Messing et al. 1991), while other literature indicates that ethanol exposure activates PKCε in intact cell systems (Miyame et al. 1997; Jiang and Ye 2003; Qi et al. 2007). Ethanol regulation of PKCε and other PKC isozymes is most likely to be indirect, due to modulation of upstream signaling pathways

that generate DAG or that lead to phosphorylation of sites necessary for full kinase activity, such as the C-terminal hydrophobic motifs of PKCε (Wallace et al. 2007) and the cPKCs (Wilkie et al. 2007).

2.2.2 Ethanol Regulation of PKC Localization

In NG108-15 neuroblastoma x glioma cells, ethanol causes translocation of PKCδ from the Golgi to the perinucleus and PKCε from the perinucleus to the cytoplasm (Gordon et al. 1997). Cytosolic translocation of PKCε has also been observed in rat cerebral cortex following acute exposure to ethanol (Kumar et al. 2006). Dopamine D2 receptor agonists stimulate translocation of PKCδ and PKCε to these same sites in NG108-15 and CHO cells stably transfected to express D2 receptors (Gordon et al. 2001), and in cultured rat VTA dopamine neurons (Yao et al. 2010). The effects of ethanol and D2 agonists are synergistic since concentrations of ethanol and agonist that do not cause translocation alone produce robust translocation when administered together (Gordon et al. 2001). Ethanol-translocated PKCε is active (Yao et al. 2008), as shown by its binding to monoclonal antibody 14E6, which specifically detects the active conformation of PKCε (Souroujon et al. 2004). Translocation of PKCε occurs together with translocation of β'COP (Gordon et al. 2001; Yao et al. 2008), a receptor for activated PKCε (εRACK; RACK2) (Csukai et al. 1997). Ethanol stimulates translocation of this complex through activation of adenosine A2a receptors, and both ethanol and D2 agonist stimulated translocation require activation of PLC, PKCε and PKA; PKA may act by promoting the activation of PLC and by phosphorylating and facilitating the translocation of εRACK (Yao et al. 2008). These results indicate considerable cross talk between PKA and PKCε in synergistic responses to ethanol and dopamine.

Such crosstalk is important in the VTA where drugs of abuse increase extracellular levels of dopamine and thereby activate D2 autoreceptors on dopaminergic neurons. This event leads to the activation of PKC (most likely PKCε) and PKA, which phosphorylate and up-regulate tyrosine hydrolase (TH) and increase production of dopamine (Yao et al. 2010). Up-regulation of TH activity by cocaine is essential for the ability of ALDH2 inhibitors to reduce cocaine self-administration in rodents (Yao et al. 2010). ALDH2 inhibitors impair metabolism of dopamine, resulting in the generation of tetrahydropapaveroline (THP), a potent inhibitor of TH, especially phosphorylated TH. It is likely that the ability of ALDH2 inhibitors to reduce ethanol self-administration (Arolfo et al. 2009) also requires PKCε and PKA-mediated up-regulation of dopamine production to generate THP, although this possibility remains to be tested.

2.2.3 PKCε Regulation of $GABA_A$ Receptors and Intoxication

The intoxicating effects of ethanol last much longer in PKCε knockout mice than in wild type mice due to impaired development of acute functional tolerance to

ethanol in the knockout (Wallace et al. 2007). Phenotypic and biochemical studies using PKCε knockout mice and selective peptide inhibitors and activators of PKCε have demonstrated that PKCε reduces the response of $GABA_A$ receptors to several positive allosteric modulators, including neurosteroids, benzodiazepines and ethanol (Hodge et al. 1999; Hodge et al. 2002). Two mechanisms appear to account for this modulation. First, PKCε phosphorylates the γ2 subunit of $GABA_A$ receptors at Ser-327, and when this site is phosphorylated, synaptic $GABA_A$ receptors show reduced activation by benzodiazepines and by ethanol (Qi et al. 2007). This phosphorylation event is important for behavior since development of acute functional tolerance is associated with increased Ser-327 phosphorylation and reduced effects of ethanol on cerebellar $GABA_A$ receptors (Qi et al. 2007; Wallace et al. 2007). Second, PKCε phosphorylates the N-ethylmaleimide-sensitive factor (NSF) at Ser-460 and Thr-461 (Chou et al. 2010). Phosphorylation at these sites increases NSF activity and binding to PKCε and alters $GABA_A$ receptor trafficking, resulting in fewer receptors at the synapse (Chou et al. 2010). Thus, inhibiting PKCε facilitates inhibitory synaptic transmission in general by increasing the density of synaptic $GABA_A$ receptors through a reduction in NSF activity and specifically enhances the positive allosteric effects of ethanol and benzodiazepines by decreasing the phosphorylation of GABA γ2 subunits.

2.2.4 PKCε and Ethanol-Induced GABA Release

Ethanol stimulates GABA release in the CeA through a mechanism that requires activation of type 1 corticotrophin releasing factor (CRF) receptors (CRF_1Rs) (Nie et al. 2004). CRF is an anxiogenic neuropeptide that is upregulated in the amygdala of ethanol-dependent rodents where it promotes excessive ethanol consumption through actions at CRF_1Rs (Chu et al. 2007; Sommer et al. 2008). Furthermore, a polymorphism in the *Crhr1* promoter that is accompanied by increased abundance of *Crhr1* transcripts in several limbic areas has been identified in Marchigian–Sardinian Preferring (msP) rats genetically selected for high alcohol preference (Hansson et al. 2007). PKCε knockout mice, which show reduced anxiety-like behavior (Hodge et al. 2002) and low levels of ethanol self-administration (Hodge et al. 1999; Olive et al. 2000), also have an ~50% reduction in levels of CRF in the CeA (Lesscher et al. 2008). Furthermore, absence or inhibition of PKCε prevents CRF or ethanol-stimulated GABA release in the CeA (Bajo et al. 2008). Therefore, PKCε is important not only for the production of CRF but also for CRF_1R signaling that controls ethanol-induced GABA release in the CeA and regulates both anxiety and ethanol consumption (Lesscher et al. 2008; Lesscher et al. 2009).

2.2.5 PKCγ and Ethanol-Mediated $GABA_A$ Receptor Trafficking

Like PKCε, PKCγ is widely expressed in the CNS (Naik et al. 2000), and also regulates $GABA_A$ receptors and behavioral responses to ethanol. In contrast to

PKCε knockout mice, PKCγ knockout mice are less sensitive to acute effects of ethanol, consume more ethanol and show impaired development of chronic tolerance to ethanol compared with wild type mice (Bowers et al. 1999; Bowers et al. 2000; Bowers and Wehner 2001). Exposure to ethanol for several hours causes internalization of α1 subunits of $GABA_A$ receptors in cerebral cortex (Kumar et al. 2003) and hippocampus (Liang et al. 2007), which may play a role in the hyperexcitablity that appears during ethanol withdrawal. In a recent study it was found that PKCγ co-immunoprecipitates with α1 subunits and this association is increased after 4 h of exposure to ethanol (Kumar et al. 2010). Treatment with short-interfering RNAs targeted against PKCγ prevented ethanol-induced decreases in the abundance of α1 subunits in cultured cortical neurons (Kumar et al. 2010), suggesting that PKCγ mediates ethanol-induced decreases in α1. However, while suggestive, these results should be viewed as preliminary since although three siRNAs were used against PKCγ in this study, it appears that they were administered together, not separately. Also, an inhibitory peptide derived from the pseudosubstrate sequence of PKCβ had no effect on α1 subunit trafficking which is puzzling since this peptide appears to also inhibit PKCγ activity (Correia et al. 2003). Finally, ethanol treatment results in the inhibition of PKCβII translocation and thus prevents proper substrate phosphorylation (Ron et al. 2000).

2.2.6 PKCδ and Sensitivity to Ethanol Intoxication

PKCδ is expressed in several brain regions that regulate ethanol intake (Merchenthaler et al. 1993; Choi et al. 2008) including the CeA (Koob et al. 1998; Finn et al. 2007; Funk et al. 2006; Primeaux et al. 2006; Moller et al. 1997; Hyytiä and Koob 1995), the hippocampus (Adell and Myers 1994; Huttunen and Myers 1987; Martin-García et al. 2007), the bed nucleus of the stria terminalis (BNST) (Walker et al. 2003; Hyytia et al. 1999) and the lateral septum (Ryabinin et al. 2008). Acute ethanol exposure alters the distribution, whereas chronic exposure increases the abundance and translocation of PKCδ in neural cell lines (Messing et al. 1991; Gordon et al. 1997), suggesting that PKCδ participates in responses to ethanol. This hypothesis has been confirmed in PKCδ knockout mice (Chou et al. 2004), which are less sensitive to the acute motor-impairing effects of ethanol (Choi et al. 2008). This resistance is most obvious at a dose of ethanol (1.5 g/kg) that produces blood ethanol concentrations of 150–240 mg/dl (32–51 mM) in mice (Gentry et al. 1983); similar blood levels impair coordination in humans (Messing 2007). These findings suggest that PKCδ is involved in neuronal signaling pathways that increase acute sensitivity to ethanol at ethanol doses that produce moderate intoxication.

2.2.7 PKCδ and Tonic GABA Currents

Although $GABA_A$ receptors are considered primary targets for ethanol, demonstration of direct effects at concentrations lower than those that produce anesthesia

has been historically difficult (Harris et al. 1997; Harris et al. 1995; Criswell and Breese 2005). However, recent electrophysiological studies have provided evidence of low dose ethanol effects at $GABA_A$ receptors in the hippocampus, cerebral cortex, NAc and CeA (Weiner and Valenzuela 2006). Evidence from electrophysiological recordings of recombinant receptors expressed in *Xenopus oocytes* (Sundstrom-Poromaa et al. 2002; Wallner et al. 2003) and of native receptors in hippocampal dentate gyrus granule cells (Wei et al. 2004; Fleming et al. 2007) suggest that $GABA_A$ receptors formed by the subunit combination of $\alpha 4\beta x\delta$ are sensitive to low (1–30 mM) concentrations of ethanol. Concentrations of 3–20 mM produce mild intoxication in humans and 17 mM is equivalent to a blood alcohol level of 80 mg/dl (Messing 2007). It must be noted, however, that some investigators have been unable to replicate these findings (Yamashita et al. 2006; Borghese et al. 2006). The basis for this discrepancy could be related partly to differences in phosphorylation state of the receptor, as discussed below.

$GABA_A$ receptors that contain δ subunits are extrasynaptic and modulate the inhibitory tone of neurons by responding to ambient GABA levels, as opposed to synaptic receptors, which contain $\gamma 2$ subunits instead of δ subunits, and provide rapid, phasic inhibition by responding to stimulated release of GABA at synapses (Farrant and Nusser 2005; Wei et al. 2003; Glykys and Mody 2007; Glykys et al. 2007). $GABA_A$ receptors that contain δ subunits have a high affinity for GABA and a slow rate of desensitization, properties that are useful for tonic regulation of inhibition. Our recent work indicates that PKCδ regulates the ethanol sensitivity of tonic inhibitory GABA currents (Choi et al. 2008). Thus, tonic currents in thalamic and hippocampal neurons of PKCδ knockout mice show no response to 30 mM ethanol. Ethanol regulation of tonic GABA current is mediated by a direct effect of ethanol on extrasynaptic $GABA_A$ receptors rather than on mechanisms that regulate extracellular concentrations of GABA (e.g. GABA transporters) since, in mouse L(tk-) fibroblasts that express $\alpha 4\beta 3\delta$ $GABA_A$ receptors, ethanol enhancement of GABA currents is also PKCδ-dependent (Choi et al. 2008). These findings suggest that PKCδ facilitates ethanol intoxication by enhancing ethanol's action at extrasynaptic $GABA_A$ receptors, possibly through phosphorylation of receptor subunits.

2.3 Extracellular Signal-Regulated Kinases (ERKs)

ERKs are serine–threonine protein kinases that are members of the mitogen-activated protein kinase (MAPK) family. There are two isoforms, p44 ERK1 and p42 ERK2, with functions that partly overlap. Both are widely expressed in limbic brain regions including in the mesolimbic dopaminergic system, amygdala and prefrontal cortex (Lein et al. 2007). ERKs are activated by a Ras–Raf–MEK signaling cascade that is activated by receptor tyrosine kinases (see section below on Receptor Tyrosine Kinases) or by calcium influx through NMDA and voltage-gated calcium channels. The function of these ion channels can be enhanced by

PKA-mediated phosphorylation resulting from activation of dopamine D1 receptors (Lu et al. 2006; Pascoli et al. 2011). Since ERK activity is increased by dopamine and glutamate receptor stimulation, it may function as a coincidence detector that combines information about rewards and contextual information during the development of addiction (Girault et al. 2007).

Since MEK phosphorylation activates ERK1 and ERK2 (ERKs), ERK activity can be indirectly assayed by measuring MEK phosphorylation of ERKs using phospho-specific antibodies. Using this approach, previous studies have reported that acute ethanol exposure (3.5 g/kg) in adult rats inhibits ERKs in the cerebral cortex, hippocampus and cerebellum (Chandler and Sutton 2005) and that continuous or intermittent exposure to ethanol vapor for 12 days also inhibits ERKs in the amygdala, cerebellum, dorsal striatum, hippocampus and PFC (Sanna et al. 2002). We recently found that acute systemic administration of 2 g/kg of ethanol to C57BL/6 mice did not alter ERK phosphorylation in the NAc (Neasta et al. 2011a). However, Ibba et al. (Ibba et al. 2009) detected ERK activation in the BNST, CeA and the NAc 15 min after intragastric gavage of 1 but not 2 g/kg ethanol; both basal and ethanol-stimulated ERK activity could be blocked by a dopamine D1 receptor antagonist. In addition, Neznanova and colleagues (Neznanova et al. 2009) observed that alcohol-preferring AA rats showed rapid and transient dephosphorylation of ERK1/2 upon acute ethanol challenge in the medial prefrontal cortex, and to a lesser degree in the nucleus accumbens, whereas alcohol non-preferring ANA rats did not. Therefore, it is possible that, under certain conditions, ethanol stimulation of dopaminergic signaling activates ERKs. In addition, ethanol can indirectly activate ERKs by up-regulating BDNF-mediated signaling in the dorsal striatum (see section below on BDNF). The mechanisms responsible for inhibition of ERKs by ethanol elsewhere are not known.

Few studies have examined ERK activity in ethanol-dependent rodents. An older report found that ERK activity is increased during ethanol withdrawal in the dorsal striatum, cerebellum and especially in the amygdala (Sanna et al. 2002). ERK activation can induce transcription of the immediate-early gene *c-fos*. Acute administration of ethanol stimulates *c-fos* in several brain regions, but only in the MeA is this ERK-dependent (Hansson et al. 2008). However, in ethanol-dependent rats, induction of *c-fos* by an ethanol challenge is inhibited in orbital frontal cortex and NAc shell through an ERK-dependent mechanism (Hansson et al. 2008), suggesting that in these brain regions ERKs are part of a homeostatic response that suppresses ethanol-induced *c-fos* expression mediated by other signaling pathways. Overall, ethanol's effects on ERK signaling are heterogeneous and depend not only on the brain region studied but also on whether animals are in an ethanol-naïve or -dependent state. The net effect of ERK signaling may be to suppress ethanol intake, since recent evidence indicates that systemic administration of the MEK inhibitor SL327 increases operant self-administration of ethanol in C57BL/6J mice (Faccidomo et al. 2009), and this inhibitory mechanism of ERKs on ethanol consumption may be mediated via BDNF (see section on BDNF).

2.4 P13K, AKT and GSK3beta

Phosphatidylinositol-3-kinase (P13K) is a lipid kinase that phosphorylates phosphatidylinositides (PtdIns) at the plasma membrane leading to the recruitment of the downstream serine and threonine kinases, 3-phosphoinositide-dependent protein kinase 1 (PDK1) and AKT, to the membrane, where PDK1 phosphorylates and activates AKT. The PI3/AKT pathway contributes to diverse biological functions such as cell survival and growth (Brazil and Hemmings 2001; Engelman 2009), and in the CNS, AKT phosphorylates the $\beta2$ subunit of the $GABA_A$ receptor leading to increased membranal localization of $\beta2$ containing $GABA_A$, thereby increasing $GABA_A$ receptor-mediated synaptic transmission (Wang et al. 2003). Interestingly, several independent investigations in flies and rodents recently indicated an important role for the P13K/AKT pathway in ethanol's actions. Specifically, inhibition of P13K in the NAc reduced binge drinking in C57BL6 mice (Cozzoli et al. 2009) and in rats (Neasta et al. 2011a). These results suggest that ethanol treatment results in the activation of P13K in the NAc, but the mechanism underlying P13K activation is not yet clear. One possibility is that ethanol activates a small Ras family G-protein upstream of P13K. Ras proteins (H-Ras, K-Ras and N-Ras) cycle between active GTP-bound and inactive GDP-bound forms. Active GTP-bound Ras interacts with several effector proteins, and among them is P13K. Interestingly, we previously observed that acute ex vivo treatment of hippocampal slices with ethanol leads to a robust activation of H-Ras (Suvarna et al. 2005).

As menioned above, AKT is activated in response to the activation of P13K. AKT activation can be measured by phosphorylation of AKT on threonine 308 and serine 473. Systemic administration of 0.75 g/kg ethanol to young adult (3-week) mice increases phosphorylation of AKT at Thr-308 in the striatum (Bjork et al. 2010). Administration of a higher dose (1.5 g/kg) increases AKT Thr-308 phosphorylation measured 45 min later in the medial prefrontal cortex, but not in the NAc of AA rats selectively bred to drink high levels of ethanol (Neznanova et al. 2009). On the other hand, we found that systemic administration of ethanol (2 g/kg) leads to the phosphorylation of AKT at both threonine 308 and serine 473 in the NAc of adult (9-week old) mice (Neasta et al. 2011a). AKT is also phosphorylated (and thus activated) in the NAc of high ethanol drinking Long-Evans rats (Neasta et al. 2011a). The activation of AKT by ethanol is likely to be an important contributor to mechanisms that lead to ethanol-drinking behaviors as the blockade of the AKT pathway within the NAc decreases excessive voluntary consumption and self-administration of ethanol in heavy drinking rats (Neasta et al. 2011a). Finally, using *Drosophila* as a model system, the Heberlein group conducted an elegant set of experiments suggesting that the P13K/AKT pathway contributes to the sensitivity of flies to the sedative actions of ethanol. Specifically, neuronal overexpression of PDK1, the catalytic subunit P13K or AKT increased the duration of ethanol sedation, whereas over expression of the dominant negative form of P13K or RNAi-mediated knockdown of AKT decreased the sensitivity of flies to the acute hypnotic

actions of ethanol (Eddison et al. 2011). Together, the studies described above strongly suggest that the P13K/AKT signaling pathway is a key contributor to mechanisms that underlie phenotypes such as excessive ethanol drinking.

The serine/threonine kinases glycogen synthase kinase-3 α and β (GSK-3α and GSK-3β) (Jope and Johnson 2004) are important and well-characterized substrates of AKT, and phosphorylation of GSK-3α on serine 21 and GSK-3β on serine 9 by AKT results in the inhibition of GSK-3 kinase activity (Jope and Johnson 2004). Neznanova et al. did not observe changes in the level of GSK-3β phosphorylation in the NAc of AA rats in response to systemic administration of ethanol, although an increase in GSK3β phosphorylation was detected in the prefrontal cortex (Neznanova et al. 2009). We recently observed that systemic administration of ethanol (2 g/kg) as well as recurring cycles of voluntary consumption of high amounts of ethanol followed by periods of withdrawal in rats lead to an increase in level of phosphorylated GSK-3α and GSK-3β in the NAc (Neasta et al. 2011a). The contribution of GSK-3α or GSK-3β inhibiton to ethanol's actions in the CNS is yet to be determined.

2.5 mTOR

A very important downstream target of AKT is the serine/threonine protein kinase, mammalian target of rapamycin (mTOR) (Hay and Sonenberg 2004). mTOR signals through two multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Hoeffer and Klann 2010). The two complexes have unique protein compositions consisting of adaptor proteins, enzymes and substrates (Hoeffer and Klann 2010). mTORC1 plays an essential role in initiating local synaptic protein translation, synaptic plasticity, learning and memory (Costa-Mattioli et al. 2009). We recently found that mTORC1 within the NAc is a novel contributor to molecular mechanisms underlying ethanol drinking (Neasta et al. 2010). Systemic administration of ethanol and high levels of ethanol intake activated mTORC1-mediated signaling in the NAc of mice and rats. In addition, levels of the AMPA receptor subunit GluR1 and the scaffolding protein Homer, two synaptic proteins whose translation is regulated by mTORC1 (Slipczuk et al. 2009), were up-regulated in these rodents (Neasta et al. 2010). Importantly, the FDA-approved inhibitor of mTORC1, rapamycin, decreased ethanol induction of Homer translation, reduced ethanol-induced locomotor sensitization and place preference, and reduced excessive ethanol intake and seeking (Neasta et al. 2010). Interestingly, the translation of postsynaptic density protein 95 (PSD-95), and the activity-regulated cytoskeleton-associated protein (Arc) have been linked to the mTORC1 pathway (Lee et al. 2005; Takei et al. 2004). Both proteins play a role in neuroadaptations underlying ethanol's actions (Carpenter-Hyland and Chandler 2006; Pandey et al. 2008b; Moonat et al. 2011; Camp et al. 2011). Therefore, it would be interesting to determine if ethanol-induced activation of mTORC1 signaling results in translation of these and other synaptic proteins.

3 Tyrosine Kinases

Tyrosine kinases are a large and diverse family of proteins that can be subdivided into two groups: receptor tyrosine kinases (RTK), and the non-receptor tyrosine kinases (NRTK). Both groups share a highly conserved kinase domain and unique protein or lipid binding domains.

3.1 Receptor Tyrsosine Kinases

RTKs are membrane-spanning receptors composed of an extracellular N-terminal region, a membrane-spanning region, and an intracellular C-terminal region, which contains the catalytic domain. Most receptors dimerize upon ligand binding allowing auto- and trans-phosphorylation to occur. Tyrosine phosphorylation at the C-terminus of the receptor serves as a docking site for adaptor proteins, which, in turn, recruit enzymes that initiate the activation of a signaling cascade. The most well-characterized ligands that interact and activate RTKs are growth factors, and in recent years several growth factors have been associated with molecular and behavioral adaptations in response to ethanol.

3.1.1 EGF

Epidermal growth factor (EGF) and its receptor (EGFR) are expressed in adult neurons in brain regions such as the hippocampus, cerebellum and cerebral cortex (Wong and Guillaud 2004). In the hippocampus EGF enhances the activity of the *N*-methyl-D-aspartate (NMDA) receptor (NMDAR) and increases long-term potentiation (LTP) in CA1 pyramidal neurons (Wong and Guillaud 2004). EGF also plays a protective role in stimulating the survival of rat cortical and dopaminergic neurons (Wong and Guillaud 2004). Recent evidence suggests a contribution of EGFR-mediated signaling to ethanol's actions in the CNS. A forward genetic screen in *Drosophila* identified mutants in the gene *happyhour*, which display a high level of resistance to ethanol intoxication (Corl et al. 2009). The protein encoded by *happyhour* shows strong homology to mammalian Ste20 family kinases and acts to inhibit EGFR-mediated activation of ERK (Corl et al. 2009). In addition, inhibitors of the EGFR signaling increase the sensitivity of both flies and mice to the intoxicating properties of ethanol (Corl et al. 2009). Interestingly, incubation of cultured cancer cells with ethanol (43 mM) produces a rapid and robust phosphorylation and activation of the EGFR and of ERK1/2, and an inhibitor of the EGFR blocks both phosphorylation events (Forsyth et al. 2010), providing a direct link between ethanol and EGFR-mediated signaling. In rats, systemic administration of EGFR inhibitors reduces voluntary consumption of ethanol but not sucrose (Corl et al. 2009). Together, these results indicate that the

EGFR is part of an evolutionary conserved signaling pathway activated by ethanol exposure that contributes to mechanisms underlying ethanol intoxication as well as consumption.

3.1.2 Insulin

Insulin is not produced in the brain, but circulating insulin crosses the blood–brain barrier. Insulin receptors (IRs) are expressed in both astrocytes and neurons in brain regions such as the hypothalamus, hippocampus, cerebellum, amygdala and cerebral cortex. Insulin's main roles in the CNS are the control of food intake and cognitive functions such as memory (Laron 2009). In *Drosophila*, activation of insulin signaling in the CNS plays a regulatory role in ethanol-mediated intoxication, as inhibition of the pathway or reduction in the function of insulin-producing cells increases the severity of fly intoxication (Corl et al. 2005). Although these results are intriguing, the role of insulin in ethanol intoxication needs to be confirmed in mammals.

3.1.3 GDNF

The glial-derived neurotrophic factor (GDNF) is a distant member of the transforming growth factor β (TGF-β) superfamily. Although GDNF was originally identified in a glial cell line (Lin et al. 1993), it is mainly expressed in neurons of the adult brain (Pochon et al. 1997; Barroso-Chinea et al. 2005). Binding of GDNF to its co-receptor, GFRα1 leads to the recruitment of the RTK, Ret, to the GFRα1-GDNF complex (Jing et al. 1996), and Ret is then activated by autophosphorylation (Durbec et al. 1996). The main signaling pathways that are downstream of Ret activation are ERK1/2, P13K and PLCγ (Airaksinen and Saarma 2002). GDNF is highly expressed in the NAc, and its receptors (Ret and GFRα1), are localized in the VTA (Trupp et al. 1997). We recently showed that dopaminergic terminals in the nucleus accumbens retrogradely transport GDNF to the VTA, where the growth factor increases the spontaneous activity of dopaminergic neurons, resulting in an increase in dopamine overflow in the NAc (Wang et al. 2010a). Accumulating evidence suggests that GDNF in the mesolimbic dopaminergic system plays an important inhibitory role in ethanol-drinking behavior. A single administration of GDNF into the VTA of Long-Evans rats results in a very rapid and sustained reduction of ethanol intake in two-bottle choice continuous access and operant self-administration paradigms (Carnicella et al. 2008, 2010; Carnicella and Ron 2009). Interestingly, GDNF's actions are specific for ethanol and are not due to a general reduction of reward or changes in locomotor activity, as the growth factor has no effect on operant self-administration of sucrose (Carnicella et al. 2008). Importantly, intra-VTA infusion of GDNF 10 min before the beginning of an operant session blocks the reacquisition of operant responding for ethanol after a period of extinction (Carnicella et al. 2008). Activation of the

GDNF pathway in the VTA results in the phosphorylation of ERKs (Carnicella et al. 2008; Wang et al. 2010a), which is required to reduce ethanol consumption, since inhibition of ERKs in the VTA blocks GDNF inhibition of ethanol self-administration (Carnicella et al. 2008). Finally, mice haploinsufficient for GDNF or its receptor, GFRα1, consume more ethanol after a period of abstinence compared with wild type littermates (Carnicella et al. 2009b). In addition, these mice exhibit increased place preference for ethanol compared with wild type mice (Carnicella et al. 2009b). These results suggest that endogenous GDNF-mediated signaling contributes to mechanisms that protect against addiction by suppressing or delaying the development of ethanol reward and limiting relapse to drinking.

3.1.4 BDNF

The brain derived neurotrophic factor (BDNF) belongs to the nerve growth factor (NGF) family of neurotrophic factors. BDNF and its receptor TrkB are widely distributed throughout the brain, and the BDNF/TrkB pathway plays an important role in neuronal proliferation, differentiation and survival, as well as synaptic plasticity (Lewin and Barde 1996; Yoshii and Constantine-Paton 2010). More recently, BDNF has been implicated in psychiatric disorders such as depression and anxiety (Martinowich et al. 2007). In addition, a growing body of literature suggests a role for BDNF in drug addiction (Ghitza et al. 2010), and we and others generated evidence that suggests a unique role for BDNF in regulating behavioral responses to ethanol. Specifically, a reduction in *BDNF* gene expression in BDNF heterozygous knockout mice (Hensler et al. 2003; McGough et al. 2004), or inhibition of the BDNF receptor TrkB (Jeanblanc et al. 2006), increases ethanol consumption and preference. Moreover, acute systemic administration of ethanol and voluntary intake of moderate amounts of ethanol, through two-bottle choice or operant self-administration paradigms, increases *BDNF* expression in the dorsal striatum of mice and rats (McGough et al. 2004; Jeanblanc et al. 2009; Logrip et al. 2009). Ethanol-mediated increases in *BDNF* mRNA result in increased synthesis of BDNF and activation of ERK (Logrip et al. 2008), and to increased expression of downstream genes, such as the dopamine D3 receptor and dynorphin (Jeanblanc et al. 2006; Logrip et al. 2008). Interestingly, BDNF in the dorsal striatum, in turn, acts as an endogenous inhibitor of ethanol consumption (McGough et al. 2004; Jeanblanc et al. 2009), and this action is localized to the dorsolateral striatum (Jeanblanc et al. 2009), a brain region associated with habit learning (Yin and Knowlton 2006). In contrast, long-term, daily ethanol intake in C57BL6 mice results in a breakdown of this protective homeostatic pathway in the dorsal striatum (Logrip et al. 2009). In addition, chronic, high levels of ethanol intake decrease cortical BDNF mRNA (Logrip et al. 2009). These results are in line with previous data showing that a decrease in cortical BDNF can be detected 24 h after withdrawal from chronic ethanol treatment (Pandey et al. 1999b).

Elegant studies by Pandey and colleagues suggest that BDNF in the CeA and MeA plays a protective role against anxiety and ethanol consumption during

ethanol withdrawal. Reducing BDNF in the amygdala increases anxiety and ethanol consumption in rats (Pandey et al. 2006; Pandey et al. 2008b). In contrast, the anxiolytic actions of ethanol are associated with increased expression of BDNF, as well as BDNF-induced expression of Arc in the CeA and MeA, and infusion of BDNF in the CeA reverses ethanol withdrawal-induced anxiety (Pandey et al. 2008b). Interestingly, BDNF mRNA and protein levels are lower in the extended amygdala of P rats compared with NP rats, which is consistent with BDNF's role in suppressing ethanol intake (Prakash et al. 2008).

Consistent with these results in rodents, Heberlein and colleagues (Heberlein et al. 2010) recently reported that the level of BDNF in the serum of alcohol-dependent patients is negatively correlated with the severity of withdrawal symptoms. In addition, a single nucleotide polymorphism (Val66Met) in the *BDNF* gene, which leads to a reduction in BDNF function (Chen et al. 2004), has been linked with an earlier onset of alcoholism (Matsushita et al. 2005), and a recent human study reported a higher risk of relapse in ethanol-dependent patients with this polymorphism (Wojnar et al. 2009).

3.2 The Src Family of Protein Tyrosine Kinases

The Src family of protein tyrosine kinases (Src PTKs) are intracellular, membrane-bound enzymes that play an important role in various cellular functions. Four members of the family are expressed in the brain (Src, Fyn, Lyn and Yes) (Kalia et al. 2004). Src and Fyn have been heavily implicated in modulation of NMDARs and synaptic plasticity (Salter and Kalia 2004). Fyn is also an important mediator of neurite outgrowth and myelination by oligodendrocytes (Beggs et al. 1994; Bodrikov et al. 2005; Brackenbury et al. 2008; Sperber et al. 2001), and has been implicated in Alzheimer's disease (Lee et al. 2004; Chin et al. 2005). Lyn was reported to interact with the Na^+/K^+ ATPase and to phosphorylate its $\alpha3$ subunit (Wang and Yu 2005). Lyn was also shown to negatively regulate NMDAR activity (Umemori et al. 2003). A link between Lyn and the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPAR) in the cerebellum has also been suggested (Hayashi and Huganir 2004). Very recently, we found that Lyn negatively regulates the release of dopamine in the mesolimbic system (Gibb et al. 2011). Although Yes is highly expressed in the basal ganglia (Walaas et al. 1993), its role in the CNS has yet to be determined.

3.2.1 Src and Fyn in Ethanol Modulation of NMDA Receptors

Interestingly, ethanol exposure results in opposing actions on the activity of Src and Fyn. Acute treatment of hippocampal neurons with ethanol inhibits Src (Suvarna et al. 2005), but activates Fyn (Yaka et al. 2003b). Src inhibition, in turn, results in the internalization of the NR2A subunit of the NMDAR (Suvarna

et al. 2005), and this mechanism contributes to the acute inhibitory actions of ethanol on channel function (Suvarna et al. 2005). In the hippocampus, ethanol stimulates Fyn-mediated phosphorylation of NR2B, which contributes to the development of acute tolerance to ethanol both ex vivo and in vivo (Ron 2004; Ron and Jurd 2005). Fyn is also activated in the dorsal striatum in response to ethanol (Wang et al. 2007; Wang et al. 2010b). Interestingly, ethanol-stimulated phosphorylation of NR2B is not observed in a structurally related brain region, the NAc (Wang et al. 2007). Furthermore, Fyn-mediated activation and NR2B phosphorylation results in long-term facilitation (LTF) of NR2B-containing NMDARs in the dorsal striatum of ethanol exposed rodents (Wang et al. 2007). This LTF of NMDAR activity is centered in the dorsomedial striatum (DMS) (Wang et al. 2010b), a brain region implicated in goal-directed behaviors (Yin et al. 2005). Importantly, repeated daily systemic administration of ethanol leads to prolonged activation of Fyn, increased NR2B phosphorylation and membrane localization of the receptor subunit in the DMS (Wang et al. 2010b). These events are associated with a long-lasting increase in the activity of the NR2B-NMDARs in this brain region (Wang et al. 2010b). Furthermore, inhibition of NR2B-NMDARs or Src family PTKs in the DMS but not the DLS or the NAc significantly decreases operant self-administration of ethanol and reduces ethanol-primed reinstatement of ethanol seeking (Wang et al. 2007; Wang et al. 2010b). Together, these results suggest that Fyn phosphorylation of NR2B and subsequent up-regulation of NMDAR function within the DMS contribute to the maladaptive synaptic changes that promote excessive ethanol intake and relapse.

In contrast to the above results, a recent study by Wu and colleagues (Wu et al. 2010) showed that tyrosine phosphorylation of NR2B in the hippocampus is markedly reduced in rats fed a liquid ethanol diet. The discrepancy between Wu's results and ours could be due to differences in paradigms. It is also important to note that the basal level of NR2B phosphorylation in Wu's study was rather high, while we did not detect a significant basal level of NR2B phosphorylation in the dorsal striatum of control rats (Wang et al. 2007; Wang et al. 2010b).

3.2.2 Lyn and Rewarding Properties of Ethanol

As mentioned above, we recently found that Lyn negatively regulates the release of dopamine in SHSY5Y human neuroblastoma cells and in the mouse NAc (Gibb et al. 2011). Acute exposure of rodents to ethanol causes a rapid increase in extracellular dopamine in the NAc (Gonzales et al. 2004), and we found that overexpression of the active form of Lyn in the VTA blocks ethanol-mediated dopamine overflow. Dopamine transmission is a contributor to ethanol reward-related behaviors (Gonzales et al. 2004), and we observed an inverse relationship between the protein level and the activity of the kinase versus the rewarding properties of ethanol (Gibb et al. 2011). Place preference for ethanol was increased in Lyn knockout mice compared with wild type littermates (Gibb et al. 2011) but was reduced in mice overexpressing an active form of Lyn in VTA neurons

compared with control mice (Gibb et al. 2011). Together, these results suggest that Lyn contributes to a mechanism that controls the extracellular levels of DA, and by doing so, the kinase reduces the rewarding properties of ethanol.

4 Scaffolding Proteins

Scaffolding proteins are a diverse group of proteins that allow for the orchestration of multiple signaling events, and provide a focal point of interaction between signaling proteins such as kinases, phosphatases, their substrates, intracellular organelles, the cytoskeletal network and the plasma membrane. Scaffolding proteins also provide platforms that allow spatially and temporally segregated events to occur. Changes in the protein–protein interactions between scaffolding proteins and their associated binding partners are potentially important consequences of neuroadaptation to ethanol. Here we review the contribution of three scaffolding proteins that play important roles in the actions of ethanol on the adult brain, although other proteins such as β-arrestin, and A-kinase anchoring proteins are likely to also be involved in mediating ethanol's effects.

4.1 RACK1

RACK1 is a scaffolding protein that is highly expressed in the CNS (Ashique et al. 2006) and was originally identified as an anchoring protein of PKCβII (Ron et al. 1994). The RACK1 amino acid sequence is characterized by seven WD40 repeats that form a seven-blade β-propeller structure (Coyle et al. 2009; Smith et al. 1999), enabling the protein to interact with a large number of binding partners including several enzymes such as Fyn kinase (Yaka et al. 2002), focal adhesion kinase (FAK) (Kiely et al. 2009), receptor protein tyrosine phosphatase μ (PTPμ) (Mourton et al. 2001), the cyclic AMP-specific phosphodiesterase isoform PDE4D5 (Yarwood et al. 1999), as well as with the intracellular tails of receptors like the insulin-like growth factor 1 receptor (IGF-1R) (Kiely et al. 2002; Hermanto et al. 2002), the inositol 1,4,5-triphosphate receptor (Patterson et al. 2004) and the NR2B subunit of NMDARs (Yaka et al. 2002). Interestingly, the intracellular compartmentalization of RACK1 changes in response to stimuli. For example, cellular stress such as hypoxia and heat shock results in RACK1 association with cytoplasmic stress granules (Arimoto et al. 2008). In contrast, upon activation of PKCβII, RACK1 shuttles active PKCβII to its site of action (Ron et al. 1999), whereas activation of PKA induces translocation of RACK1 to the nucleus (Yaka et al. 2003a; He et al. 2010). Exposure of cells to ethanol changes the intracellular localization of RACK1 via a mechanism that requires activation of PKA (Ron et al. 2000; He et al. 2002; Yaka et al. 2003b; Wang et al. 2007). One of the consequences of RACK1 nuclear localization is the inhibition of PKCβII translocation which was observed both in cultured cells and in vivo

(Ron et al. 2000). Furthermore, under basal conditions, RACK1 interacts with and localizes Fyn kinase in close proximity to the NR2B subunit of the NMDAR, but inhibits the ability of Fyn to phosphorylate the channel until the appropriate signal occurs (Yaka et al. 2002; Yaka et al. 2003a; Thornton et al. 2004). Formation of this tri-molecular complex is not ubiquitous in the brain; it is found in hippocampus and dorsal striatum, but not in the cerebral cortex or NAc (Yaka et al. 2003a; Wang et al. 2007) (see also section on Src, Fyn and modulation of NMDAR function in response to ethanol). In the hippocampus and dorsal striatum, acute ex vivo ethanol treatment releases RACK1 from the NMDAR complex, which enables Fyn to phosphorylate NR2B (Yaka et al. 2003b; Wang et al. 2007). In addition, ethanol-stimulated translocation of RACK1 to the nucleus increases expression of *BDNF* in hippocampal and dorsal striatal neurons (McGough et al. 2004). In SHSY5Y cells, nuclear RACK1 localizes at the promoter IV region of the *BDNF* gene, resulting in chromatin modifications that lead to promoter-controlled *BDNF* exon IV transcription (He et al. 2010). It will be of great interest to determine if RACK1-dependent epigenetic modulation of *BDNF* expression contributes to ethanol's actions in the brain (see also section on Epigenetic regulation of gene expression). Finally, in vivo evidence suggests that RACK1-mediated increases in BDNF levels in the dorsal striatum are part of a homeostatic pathway that regulates behaviors such as voluntary ethanol intake and ethanol sensitization (McGough et al. 2004; Jeanblanc et al. 2006) (see also section on Receptor Tyrosine Kinases). In summary, RACK1 provides an example of how changes in the compartmentalization of a scaffolding protein, as well as modifications in protein–protein interactions, can lead to the inhibition or activation of numerous signaling pathways in response to ethanol exposure.

4.2 PSD-95

The postsynaptic density protein of 95 kDa (PSD-95) is a core scaffolding protein that clusters NMDARs at glutamatergic synapses, connecting the receptors to the cytoskeleton and to signaling proteins that regulate channel function (Kim and Sheng 2004). PSD-95 has been implicated in synaptic plasticity underlying learning and memory (Kim and Sheng 2004). A recent study shows that PSD-95 knockout mice exhibit greater signs of ethanol intoxication and show less voluntary ethanol intake than wild type littermates (Camp et al. 2011). Although both genotypes showed similar levels of ethanol preference, wild type, but not PSD-95 knockout mice, maintained their preference for ethanol when tested 14 days later. Surprisingly, the deficits attributed to deletion of PSD-95 do not seem to involve altered NMDAR function since MK801, an NMDAR antagonist-enhanced ethanol intoxication to a similar extent in both genotypes (Camp et al. 2011). These results suggest that PSD-95 contributes to the level of ethanol intoxication, which can influence ethanol intake. In addition, these results imply that PSD-95 contributes to reward memory. However, the mechanism by which PSD-95 contributes to ethanol's actions in vivo has yet to be unraveled.

4.3 Homer

Homer proteins are structurally related scaffolding proteins that are the products of three independent genes, Homer1, 2 and 3 (Fagni et al. 2002). These genes can give rise to constitutively expressed long isoforms (Homer1b, c, d, Homer 2a, b and Homer3) and an immediate-early gene (short) isoform (Homer1a) (Soloviev et al. 2000). The long Homer isoforms contain a coil–coil domain and leucine zipper motifs allowing them to assemble as multimers (Hayashi et al. 2006). Homer proteins contain the protein–protein interaction binding motif Enabled/vasodilator-stimulated phosphoprotein homology 1 (EVH1) that enables the direct interaction of homers with various proteins. Homer proteins connect ion channels and receptors to intracellular calcium storage, the cytoskeleton (Thomas 2002; Sala et al. 2001), and to various signaling cascades including ERK (Mao et al. 2005) and P13K (Rong et al. 2003).

Several studies by Szumlinski and colleagues indicate that the Homer2 isoform plays an important role in ethanol's actions. Consumption of high levels of ethanol increases Homer2 expression in the NAc of mice, and this increase persists even 2 months after the last ethanol-drinking session (Klugmann and Szumlinski 2008; Cozzoli et al. 2009). Our finding that high levels of ethanol intake increase Homer proteins in the NAc via mTORC1 (Neasta et al. 2010) (see section on mTOR) may provide a mechanism for ethanol-mediated induction of Homer2 protein levels, although the antibodies we used did not differentiate between the long isoforms of Homer. Homer2 knockout mice consume less ethanol than wild type mice in a two-bottle choice continuous access paradigm, and do not develop ethanol place preference or locomotor sensitization to ethanol (Szumlinski et al. 2005). Homer2 knockout mice instead show ethanol place aversion and an increased hypnotic response to high doses of ethanol compared with wild type mice. In addition, Homer2 knockout mice do not show certain characteristic neurochemical changes associated with repeated ethanol administration (Szumlinski et al. 2005). In line with these findings, knockdown of Homer2 in the shell of the NAc reduces binge drinking in C57BL/6 mice (Cozzoli et al. 2009) and both the behavioral and neurochemical abnormalities in Homer2 knockout mice can be rescued by an administration of an adeno-associated virus (AAV) expressing Homer2 into the NAc (Klugmann and Szumlinski 2008). Interestingly, the contribution of the Homer gene to ethanol's actions has also been observed in *Drosophila*; flies lacking the Homer gene show increased sensitivity to the sedative actions of ethanol and do not develop acute tolerance to ethanol (Urizar et al. 2007).

5 Epigenetic Regulation of Gene Expression

Transcription factors and other regulatory proteins regulate gene expression by binding to specific sites in the genome. Layered on top of this process are epigenetic mechanisms that control the way genomic DNA is packaged into

chromatin and regulate access of transcription factors to target DNA sequences. These mechanisms regulate DNA methylation, covalent modification of histones and positioning of nucleosomes, and have the potential to produce long-lasting changes in gene expression that are self-perpetuating in the absence of the signals that initiate them. Chromatin changes may be transient or long lasting, mitotically transmissible, and in some cases inherited through meiosis to the next generation (Youngson and Whitelaw 2008; Dulac 2010). Epigenetic mechanisms have recently become topics of intense interest in the addiction field since they could produce persistent neuroadaptations that underlie drug tolerance and addiction (Tsankova et al. 2007). This field of research is still young and ethanol-induced modifications of histone and DNA are just now being identified. Here we describe a few recent examples in the nervous system.

5.1 DNA Methylation

Methylation of cytosine bases in DNA is mainly restricted to CpG dinucleotides and plays an important role in silencing of genes, inactivation of one X chromosome in females and in genomic imprinting of parental alleles. Proteins such as MECP2, which contain a methyl-CpG-binding domain (MBD) can inhibit, or in some cases facilitate, gene expression by binding to methylated CpG islands commonly located in gene promoter regions. DNA methyltransferases (DNMTs) catalyze DNA methylation and are critical for normal development. The enzymes are also expressed in post-mitotic neurons and recent evidence using DNMT inhibitors suggests that DNMTs mediate neuronal plasticity associated with memory (Miller and Sweatt 2007).

Although DNA methylation had been thought to be stable once established, recent evidence indicates that it can be dynamically regulated. For example, in response to maternal care, the glucocorticoid receptor promoter undergoes demethylation leading to increased expression of the receptor and a reduced response of the hypothalamic-pituitary axis to stress in the offspring (Szyf et al. 2005). The mechanisms responsible for DNA demethylation in adult neurons are not yet known.

In humans, chronic alcoholism is associated with increased circulating levels of homocysteine, probably due to impaired homocysteine metabolism (Bleich and Hillemacher 2009). Homocysteine is methylated to yield methionine, which can be metaboilized to S-adenosyl-L-methionine (SAM); SAM is a methyl group donor for DNMTs. This mechanism may explain why chronic exposure to alcohol can lead to hypermethylation and transcriptional silencing of some genes. For example, maternal ingestion of ethanol before or during gestation in mice leads to hypermethylation and reduced gene expression at the epigentically sensitive Agouti viable yellow (A_{vy}) allele of the *Agouti* gene in the offspring (Kaminen-Ahola et al. 2010). Hypermethylation has also been found in cell cycle genes of

ethanol exposed neural stem cells (Hicks et al. 2010). These findings suggest that DNA hypermethylation contributes to teratogenic effects of alcohol.

Ethanol exposure can also lead to demethylation and increased expression of certain genes, such as the *NR2B* subunit of NMDA receptors. Specifically, the abundance of the NR2B subunit is persistently up-regulated in C57BL/6 J mouse cortical neurons following chronic intermittent ethanol (CIE) treatment and, a recent analysis suggests that the mechanism involves DNA demethylation of CpG islands within the 5′ regulatory region of the *Grin2b* gene (Qiang et al. 2010). CIE treatment decreased the association of MeCP2 with chomatin and with regulatory regions of the gene. Conversely, methylation of these regions in vitro decreased binding of the CREB transcription factor to the *Grin2b* promoter. Treatment with SAM to promote DNA methylation prevented CIE-induced demethylation of the *Grin2b* promoter and CIE-induced increases in NR2B mRNA. The mechanism for this effect appeared to involve a CIE-mediated decrease in the level of mRNA for the DNA methyltransferase Dnmt1 that persists for at least 5 days after ethanol treatment; however, how this decrease occurs is not yet known.

5.2 *Histone Acetylation and Up-Regulation of Neuropeptide Y*

Covalent modification of histone is another mechanism for epigenetic regulation of gene expression. Modifications occur at N-terminal tail regions of histones and alter histone-DNA and histone–histone binding. Identified modifications include acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation and SUMOylation (Kouzarides 2007). Most is known about histone acetylation in regulating neuronal gene expression. Histone acetyltransferases (HATs) add acetyl groups to specific lysine residues, which relaxes local chromatin structure and permits transcription factor binding to DNA (Tsankova et al. 2007). For example, the CREB binding protein (CBP) is a HAT and its recruitment by CREB facilitates CREB-mediated gene expression. Conversely, histone deacetylases (HDACs) remove acetyl groups, thereby promoting chromatin condensation and decreasing transcription.

Recently, Pandey and colleagues (Pandey et al. 2008a) reported that the anxiolytic effect of ethanol is associated with increased levels of CBP, histone acetylation and NPY in the rat CeA and MeA, whereas ethanol withdrawal is associated with increased anxiety-like behavior and decreased CBP, histone acetylation and NPY in these brain regions. To investigate whether changes in histone acetylation are causally related to anxiety and NPY expression, the authors treated ethanol-withdrawn rats with trichostatin A (TSA), an HDAC inhibitor. TSA restored histone acetylation and NPY expression, and reduced anxiety-like behavior in rats undergoing ethanol withdrawal. These findings suggest a link between these events, but these results should be viewed with caution given the action of HDACs on other genes, as well as the limited specificity of TSA (Dulac 2010).

5.3 MicroRNA

MicroRNAs (miRNAs) are a large family of non-coding RNAs that may control translation from as many as 60% of all protein-coding transcripts (Hicks et al. 2010). Primary miRNA transcripts show internal complementarity and thus adopt a stem-loop structure. These precursors are processed by ribonucleases to form mature 21–23 bp miRNAs that form miRNA-induced silencing complexes (miRISCs) by associating with the proteins Argonaute and GW182 [glycine-tryptophan (GW) repeat-containing protein of 182 kDa]. miRNAs regulate protein synthesis by base-pairing to target mRNAs, most commonly at their 3′-untranslated region, allowing miRISC-mediated repression of translation, or induction of deadenylation and degradation of mRNA.

Recent studies have documented ethanol-induced changes in up to 3% of miRNAs in models of alcohol-induced liver disease and teratogenesis, but less is known about ethanol regulation of miRNA in the adult nervous system (Miranda et al. 2010). A recent, in-depth study by Pietrzykowski and colleagues identified miR-9 as a key regulator of ethanol-sensitive BK channel splice variants that contributes to ethanol tolerance (Pietrzykowski et al. 2008). The BK channel is a high conductance calcium- and voltage-dependent potassium channel that is potentiated by ethanol (Treistman and Martin 2009). In the rat supraoptic nucleus and striatum these channels develop tolerance to ethanol, resulting from decreased ethanol sensitivity and reduced channel density. The decrease in BK channel density is associated with decreased mRNA encoding the main pore-forming subunit of the channel, and involving, in particular, those mRNA splice variants that recognize miR-9 and encode for subunits that are most ethanol-sensitive. Analysis of other predicted miR-9 target transcripts with a known role in ethanol's actions revealed 8 whose expression was decreased and 2 whose expression was increased by exposure to 20 mM ethanol for 15 min. These targets encode several proteins of interest for understanding addiction, such as clock, the dopamine D2 receptor, the $\beta 2$ subunit of $GABA_A$ receptors and the $\beta 1$ subunit of voltage-gated calcium channels (Pietrzykowski et al. 2008). How ethanol rapidly increases levels of miR-9 is not known, but may involve increased expression or processing of its precursor.

6 Summary

In this chapter, we covered progress that has been made on elucidating signaling pathways such as those involving PLC/PKC and P13K/AKT/mTORC1 that underlie or maintain behaviors associated with alcohol use disorders, as well as cascades initiated by growth factors such as GDNF that act in the opposite direction. We emphasized the role of signaling molecules such as protein kinases that control post-translational modifications in response to alcohol exposure in

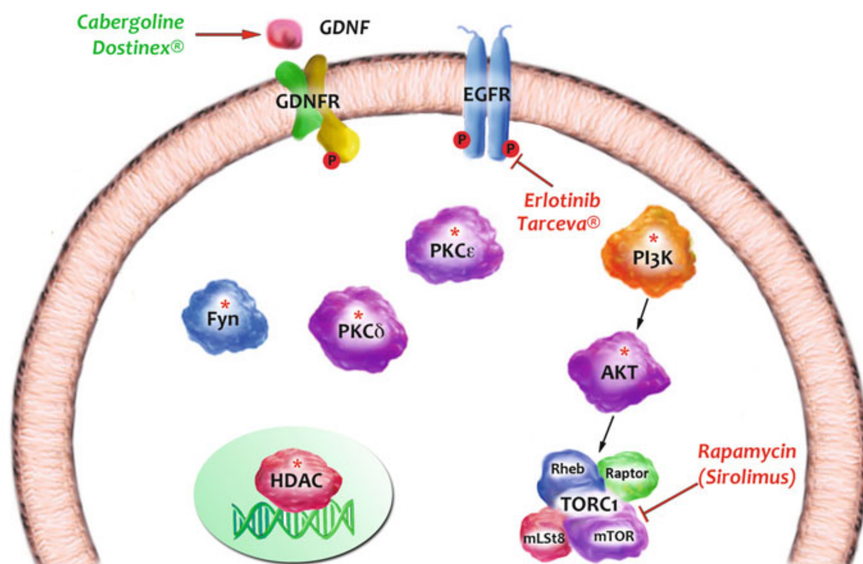

Fig. 1 Ethanol alters the function of membrane and intracellular enzymes. Inhibitors or up-regulators of these targets can be developed as novel therapeutics for the treatment of alcohol use disorders. Depicted are examples of such targets. *Asterisks* denote targets for which inhibitors are in development. *Red* depicts FDA-approved kinase inhibitors. *Green* depicts an FDA-approved medication that up-regulates the expression of a protective gene

rodents, as protein kinases hold great promise as therapeutic targets for CNS diseases (Chico et al. 2009). Most research and development efforts on kinases as drug targets have been focused on oncology. However, signaling cascades are shared across cell types, and information generated in other systems can be of potential use for alcohol use disorders. For example, the EGFR inhibitor, TARCEVA® (Erlotinib), is used to treat non-small-cell lung cancer, yet was reported by Heberlein and colleagues to reduce voluntary consumption of ethanol (Corl et al. 2009) (Fig. 1). Another example is the mTORC1 inhibitor, rapamycin (sirolimus), which is used clinically to prevent rejection in organ transplantation yet was recently found in preclinical rodent models to reduce excessive ethanol intake and seeking (Neasta et al. 2010) (Fig. 1). In addition to mTORC1, its upstream kinase activators AKT and P13K, which show promise as potential drug targets in rodent models (Cozzoli et al. 2009; Neasta et al. 2011b) (Fig. 1), are currently being targeted by pharmaceutical companies for the treatment of various types of cancers (LoPiccolo et al. 2008). In addition, other signaling targets that are potentially of great interest for drug development are PKCε, PKCδ and Fyn (Hodge et al. 1999; Khasar et al. 1999; McMahon et al. 2000; Yaka et al. 2003b; Yaka et al. 2003c; Wang et al. 2007; Wang et al. 2010b), as well as HDAC (Pandey et al. 2008a) (Fig. 1). Of interest are the very recent advances that are being made in the development of small molecules that disrupt protein–protein interactions between

signaling and scaffolding proteins (Arkin and Whitty 2009; Blazer and Neubig 2009) that may allow a high degree of desirable specificity in inhibitor action. Another intriguing possibility is the use of FDA-approved drugs such as cabergoline (Dostinex), which are approved for other indications but show promise in preclinical rodent models (Carnicella et al. 2009a) (Fig. 1). In summary, the examples described above put forward the possibility of developing small-molecule inhibitors or activators of specific signaling molecules as novel treatments for alcohol use disorders.

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Neurocircuitry Involved in the Development of Alcohol Addiction: The Dopamine System and its Access Points

Bo Söderpalm and Mia Ericson

Abstract The brain reward system, and especially the mesolimbic dopamine pathway, plays a major role in drug reinforcement and is most likely involved in the development of drug addiction. All major drugs of abuse, including ethanol, acutely activate the mesolimbic dopamine system. Both this acute drug-induced dopamine elevation, the dopamine elevations observed after presentations of drug-associated stimuli and alterations of dopamine function induced by chronic drug administration are of importance. Whereas the mechanisms of actions for central stimulants, opioids and nicotine in their dopamine activating effects are fairly well established, the corresponding mechanisms with respect to ethanol have been elusive. Here we review the actions of ethanol in the mesolimbic dopamine system, focusing on ethanol's interaction with ligand-gated ion-channel receptors, opiate receptors, the ghrelin system and the possible involvement of acetaldehyde. Preclinical studies have provided the opportunity to dissect these interactions in some detail and although we do not fully comprehend the actions of ethanol there have been some great advances resulting in increased knowledge of the complexity of ethanol's mechanism of action in this system.

Keywords Dopamine · Ethanol · Ligand-gated ion-channels · Opiate receptors · Ghrelin

B. Söderpalm (✉) · M. Ericson
Addiction Biology Unit, Section of Psychiatry and Neurochemistry,
Institute of Neuroscience and Physiology Sahlgrenska Academy,
University of Gothenburg, Gothenburg, Sweden
e-mail: bo.soderpalm@neuro.gu.se

B. Söderpalm
Beroendekliniken, Sahlgrenska University Hospital,
Blå Stråket 15, 413 45 Gothenburg, Sweden

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Contents

1 Brain Dopamine Systems 128
2 Ethanol and Brain Dopamine Systems 129
3 Ethanol, Acetaldehyde and the Mesolimbic Dopamine System 131
4 Ethanol and Cystein-Loop Ligand-Gated Ion Channels 132
4.1 Ethanol and Nicotinic Acetylcholine Receptors 133
4.2 Ethanol and Glycine Receptors 133
4.3 Ethanol and $GABA_A$, 5-HT_3 and NMDA Receptors 134
5 Ethanol, nAChR and the Mesolimbic Dopamine System 135
6 Ethanol, GlyR and the Mesolimbic Dopamine System 138
7 Ethanol, the Mesolimbic Dopamine System and 5-HT_3 Receptors 140
8 Ethanol, the Mesolimbic Dopamine System and $GABA_A$ Receptors 141
9 Ethanol, the Mesolimbic Dopamine System and Glutamate Receptors 141
10 Ethanol, the Mesolimbic Dopamine System and Opioid Receptors 142
11 Ethanol, the Mesolimbic Dopamine System and Ghrelin 143
12 Alcohol Relapse-Preventing Drugs and the Mesolimbic Dopamine System 144
13 Summary and Implications 145
References 148

Abbreviations

5-HT	Serotonin
ACTH	Adrenocorticotrophic hormone
ADHD	Attention-Deficit/Hyperactivity Disorder
AMPA	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
GABA	Gamma-aminobutyric acid
GHS-R	Growth hormone secretagogue receptor
GlyR	Glycine receptor
nAc	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptor
NMDA	n-methyl-d-aspartate
NR1/2	NMDA receptor one/two
rt-PCR	Reverse transcriptase polymerase chain reaction
VTA	Ventral tegmental area

1 Brain Dopamine Systems

In the late 1950s, dopamine was discovered as a brain neurotransmitter in its own right (Carlsson et al. 1957, 1958). In the 1960s (Dahlström and Fuxe 1964) and onwards histochemical techniques enabling visualization of dopamine containing neurons revealed the presence of four different dopamine systems, (1) the nigro-striatal dopamine system originating in the substantia nigra in the mesencephalon and projecting to the dorsal striatum (caudate-putamen), (2) the mesolimbic

dopamine system originating in the ventral tegmental area (VTA) close to the substantia nigra and projecting to the ventral striatum, the bed nucleus of stria terminalis, septum, the amygdala and the hippocampus, (3) the mesocortical dopamine system projecting from the VTA to the frontal cortex, and (4) the tuberoinfendibular dopamine system controlling prolactine release from lactotrophs in the hypophysis (Kandel et al. 2000). Lately, yet another dopamine system located to the thalamus has been discovered (Sánchez-González et al. 2005). This system is considerably more developed in monkeys and humans than in rodents and has been implicated in the pathophysiology of schizophrenia (García-Cabezas et al. 2009; Lisman et al. 2010). Several G-protein coupled receptors associated with the dopamine system (dopamine D1–D5) have been discovered and the dopamine system and its receptors have been shown to be involved in the modulation of a number of important aspects of behavior, such as movement and posture, instrumental learning, reward and motivation as well as cognitive functions, e.g. working memory, executive functions and attention, and malfunctioning of the dopamine system has been connected to a range of devastating human disorders, e.g. Parkinson's disease, schizophrenia and other psychoses, mania/ depression, ADHD and drug and alcohol addiction (Kandel et al. 2000). Some very important pharmacotherapies modulate the function of the dopamine systems, such as L-DOPA for Parkinson's disease, neuroleptics for schizophrenia and central stimulants for ADHD, underlining the importance of this neurotransmitter system and motivating further in-depth studies of it across all its assigned functions. Indeed, despite the discoveries of a number of other neurotransmitters, e.g. amino acids and neuropeptides, the profound importance of the catecholamine dopamine for proper brain functioning has become ever clearer.

2 Ethanol and Brain Dopamine Systems

Already in the 60 and 70 s ethanol, as well as all other major drugs of abuse, were shown to activate brain catecholamine systems (Corrodi et al. 1966; Carlsson and Lindqvist 1973) and this activation may be reflected in enhanced psychomotor activity, expressed as locomotor stimulation in experimental animals (Carlsson et al. 1972) and in social interaction, talkativeness etc. in man (Ahlenius et al. 1973). In the late seventies it was suggested that these drug effects are related to the rewarding properties of addictive drugs and that studies of their neurochemical background may offer new avenues for pharmacological treatment of e.g. alcoholism (Engel 1977; Engel and Carlsson 1977). All drugs of abuse have subsequently been demonstrated to increase dopamine release in several brain regions, but the effect appears to be most pronounced in the nucleus accumbens (nAc), a major target of the mesolimbic dopamine system and a central component of the brain reward system (DiChiara and Imperato 1985, 1988; Imperato et al. 1986). The mesolimbic dopamine system has been extensively reviewed many times, and it is beyond the scope of the present article to elaborate on all the different aspects

of its proposed function(s). The interested reader is referred to such reviews (e.g. Wise 1987; Wise and Rompre 1989; Koob 1992; Robinson and Berridge 1993; Spanagel and Weiss 1999). Interestingly, however, the function of the mesolimbic dopamine system is severely impaired upon cessation of subchronic exposure to all drugs of abuse, including ethanol, and this decrease of function has been associated with enhanced drug intake, presumably as a means for the organism to compensate for the reduced baseline function of the system (Diana et al. 1993; Epping-Jordan et al. 1998; Ahmed and Koob 2005).

That systemic ethanol injections increase extracellular dopamine levels in the rat and mouse nAc has been demonstrated by means of in vivo microdialysis and in vivo voltammetry by a number of investigators in several different laboratories (e.g. DiChiara and Imperato 1985; Imperato et al. 1986; Blomqvist et al. 1993, 1997; Diana et al. 1993). It has also been shown that voluntary consumption of ethanol increases accumbal dopamine levels. These increases correlate with blood ethanol levels and restore the dopamine deficits associated with ethanol withdrawal in dependent rats (Weiss et al. 1993, 1996; Ericson et al. 1998; Molander et al. 2005). Furthermore, evidence obtained in rats show that dopamine levels in the nAc increase also in anticipation of ethanol consumption, indicating a potentially important role for accumbal dopamine in relapse processes (Weiss et al. 1993, Katner et al. 1996; Katner and Weiss 1999; Melendez et al. 2002; Löf et al. 2007b). Several animal studies have indicated that blockade of dopamine receptors in the nAc reduces alcohol consumption (Levy et al. 1991; Rassnick et al. 1992; Hodge et al. 1997; Kaczmarek and Kiefer 2000; Czachowski et al. 2001) and that alcohol preference is markedly reduced in dopamine D2 knock-out mice (Phillips et al. 1998), but there are also studies showing that extensive lesioning of the dopamine system fails to decrease ethanol consumption once established (Kiianmaa et al. 1979; Rassnick et al. 1993; Fahlke et al. 1994; Koistinen et al. 2001), even though the establishment of the behavior may be compromised (Ikemoto et al. 1997, see however Lyness and Smith 1992; Koistinen et al. 2001; Shoemaker et al. 2002). Some studies indicate the opposite, i.e. that lesioning of dopamine neurons or targets in the nAc increases an established ethanol consumption (Quarfordt et al. 1991; Hansen et al. 1995), results which however are in consonance with the suggestion that decreased dopamine tone in this area drives drug intake (Diana et al. 1993; George et al. 1995; Epping-Jordan et al. 1998; Ahmed and Koob 2005).

Recently, advanced neuroimaging techniques have demonstrated that ethanol enhances extracellular dopamine levels also in the human ventral striatum and that this effect correlates with subjective estimates of euphoria, stimulation, etc. (Boileau et al. 2003; Yoder et al. 2007; Urban et al. 2010; Ramchandani et al. 2011). Studies in humans also show alterations of brain dopamine systems in abstinent alcoholics, in which reduced dopamine synthesis, reduced numbers of dopamine D2/3 receptors, and reduced displacement of binding to these receptors after challenge with the dopamine reuptake blocker methylphenidate or amphetamine have been demonstrated (Volkow et al. 1996, 2002, 2007; Heinz et al. 2005; Martinez et al. 2005), where some of these measures correlate with craving and

subsequent relapse to alcohol drinking (Heinz et al. 2005). The latter findings indicate reduced baseline dopamine activity in alcoholics, which is in line with what has been observed in animals predisposed to alcohol drinking or that have been exposed to chronic alcohol.

Despite the above data indicating the involvement of the mesolimbic dopamine system in ethanol consumption and in alcoholism there have been relatively few studies aimed at elucidating the molecular mechanisms by which ethanol activates the mesolimbic dopamine system. An identification of these will probably be of importance for finding new pharmacological means to combat alcoholism, perhaps without interfering with the general function of the brain reward system. In this context ethanol's well-established ability to interact with members of the cystein-loop ligand-gated ion channels is of considerable interest (e.g. Grant 1994). Below these interactions will be outlined as well as the tentative involvement of the ionotropic n-methyl-d-aspartate (NMDA) receptor, which ethanol also directly interacts with, as well as the endogenous opioid and ghrelin systems, which have been implicated in ethanol's activation of the mesolimbic dopamine system. Nicotinic acetylcholine (nAChR) and glycine receptors (GlyR) will be discussed in some more detail than the other receptors/systems, since these receptors have been the main focus of the authors' research since several years. However, first the possibility that ethanol's interaction with the dopamine system is mediated via its metabolite acetaldehyde rather than by ethanol itself should be considered.

3 Ethanol, Acetaldehyde and the Mesolimbic Dopamine System

Acetaldehyde, the first metabolite of ethanol, has traditionally been regarded as a mediator mainly of the aversive effects of alcohol, a notion reinforced by genetic findings indicating that polymorphisms producing increased acetaldehyde levels after ethanol ingestion are associated with a reduced risk of developing alcoholism (Edenberg 2007). In spite of this, evidence obtained in experimental animals suggests that acetaldehyde may play an important role in the rewarding, motivational and addictive properties of alcohol (Ortiz et al. 1974; Foddai et al. 2004; Melis et al. 2007) (see Quertemont et al. 2005 for review), and it has been proposed that acetaldehyde is aversive when acting in the periphery but rewarding in the central nervous system. Thus acetaldehyde induces conditioned place preference in rats (Smith et al. 1984) and is self-administered both intravenously (Myers et al. 1982) and into the cerebral ventricles (Amit et al. 1977). It has also been shown that genetically selected alcohol-preferring rats operate to obtain acetaldehyde directly into the posterior VTA (Rodd-Hendricks et al. 2002).

Considering these behavioral effects, the issue of whether the mesolimbic dopamine system can be activated by acetaldehyde and/or by the levels of acetaldehyde produced by systemic ethanol becomes very important. Indeed, administration of acetaldehyde in the VTA has been demonstrated to increase dopaminergic neuronal activity in vivo (Foddai et al. 2004) and in vitro

(Melis et al. 2007), suggesting that acetaldehyde, by increasing the possibility of neurotransmitter release from VTA dopaminergic neurons, could enhance nAc dopamine release. This is supported also by a microdialysis experiment showing that administration of 75 μM acetaldehyde for 15 min in the posterior VTA increases extracellular dopamine levels in the nAc (Melis et al. 2007). It has moreover been demonstrated that ethanol induced increases in VTA neuronal activity and dopamine release can be prevented by local coapplication of a catalase inhibitor (Melis et al. 2007), the key enzyme for ethanol oxidation in the brain (Zimatkin et al. 2006). These findings would suggest that the mesolimic dopamine activating and positive reinforcing effects of ethanol may in fact be mediated by acetaldehyde. However, the question of whether brain acetaldehyde levels produced by physiologically relevant concentrations of ethanol are sufficient to produce any pharmacological or behavioral effects relevant to reward and addiction, remains controversial (see (Deitrich 2004) for review).

By what mechanism acetaldehyde would enhance dopamine neuronal activity is not clear. Interestingly, electrophysiological studies have shown that acetaldehyde significantly enhances alpha-1-GlyR currents in *Xenopus laevis* oocytes (Mascia et al. 2001), which, together with other experimental findings (see below), could indicate that acetaldehyde might modulate dopamine output by interacting with GlyRs.

4 Ethanol and Cystein-Loop Ligand-Gated Ion Channels

Cysteine-loop ligand-gated ion channels are a family of receptors composed of five subunit proteins forming an ion channel passing through the neuronal cell membrane. The subunits appear in many forms and show different degrees of homology, which sorts them into different subgroups of receptors and subunits of receptors. Nicotinic acetylcholine receptors (nAChRs), GlyRs, $GABA_A$ receptors and $5\text{-}HT_3$ receptors all are cysteine-loop ion channels. Interestingly, it has been demonstrated both in vitro and in vivo that ethanol in relevant concentrations (10–100 mM) functionally interferes with many of these receptors, both with respect to their activation and their deactivation (desensitization). The nature of the interference appears to be determined i.a. by the subunit composition of the receptor in question (cf. Grant 1994). Experiments applying alcohols with different chain-lengths have revealed different cut-off values with respect to the carbon number for different receptor types indicating some kind of interaction site that constrains the size of the interacting molecule. These experiments also suggest that the interaction of ethanol with these receptors does not derive from lipid bilayer perturbation, which should not be limited by the size of the alcohol, but instead probably involves an interaction with the proteins themselves.

4.1 Ethanol and Nicotinic Acetylcholine Receptors

The nAChRs are composed of five subunit proteins forming a cation channel passing through the neuronal cell membrane (for review, cf. Lukas et al. 1999). Acetylcholine and nicotine bind to the receptor and thereby regulate the permeability of the ion channel, neuronal activity and transmitter release. The different subunits (α_2–α_{10}, β_2–β_4) are differentially expressed in the brain. Nicotine produces its pharmacological effects via nAChRs, especially via $\alpha_4\beta_2$* and α_7 homomeric receptors, which are the most abundant nAChRs in the brain. nAChRs are also present in the peripheral nervous system, both in ganglia and at the motor endplate. In vitro and in vivo the nAChR may undergo desensitization upon agonist exposure and the pharmacodynamic effects of nicotine may derive both from activation and desensitization of nAChRs.

Already in 1967, experiments performed in the frog indicated that ethanol interacts with nAChRs in the peripheral nervous system (Inoue and Frank 1967; Okada 1967), and in 1980, based on similar electrophysiologcal studies, it was suggested that ethanol's interaction with nAChRs could be involved in the addictive properties of the drug (Bradley et al. 1980). A number of studies in *Torpedo* later showed that ethanol affects nAChRs with respect both to activation and deactivation (e.g. Ei-Fakahany et al. 1983; Forman et al. 1989; Wu and Miller 1994). A fairly large body of literature has demonstrated that ethanol interacts directly also with central neuronal nAChRs in vivo (Criswell et al. 1993; Frohlich et al. 1994; Yang et al. 1999a, b) as well as in neuronal cell cultures and in different cells expressing human or rat nAChRs (Yu et al. 1996; Covernton and Connolly 1997; Aistrup et al. 1999; Cardoso et al. 1999; Zuo et al. 2001; Borghese et al. 2003a, b). The nature of the interaction with nAChRs is determined by the type of receptor expressed; response potentiation is observed in some subtypes (e.g. $\alpha_2\beta_4$, $\alpha_4\beta_4$, $\alpha_2\beta_2$, $\alpha_4\beta_2$ (human); $\alpha_3\beta_4$, $\alpha_2\beta_4$ (rat), whereas antagonism (e.g. α_7 oligomeres (human and rat)), or no effect is observed in others. Ethanol is a nAChR co-agonist rather than an agonist in its own right, i.e. it potentiates the acetylcholine effect but does not activate the receptor by itself (Marzalec et al. 1999). Chronic ethanol administration influences radioligand binding of nicotine to nAChRs, with varying results across studies and depending on the brain region investigated (Yoshida et al. 1982; Nordberg et al. 1985; Burch et al. 1988; Collins et al. 1988; Penland et al. 2001; Rezvani and Levin 2002).

4.2 Ethanol and Glycine Receptors

The strychnine-sensitive GlyR is a pentameric membrane protein composed of ligand-binding α- and structural β-subunits (Betz 1992; Grudzinska et al. 2005). Four α-subunits (α_1–α_4) and one β-subunit have been identified (Lynch 2004; Kirsch 2006), which are unevenly distributed in the adult central nervous system

(Malosio et al. 1991; Kuhse et al. 1995). In the hippocampus, GlyRs are e.g. thought to be mainly α_2 homomers (with small amounts of detected α_3 and β subunits) and these exert their function extrasynaptically (Brackmann et al. 2004). Tonic activation of these α_2 GlyRs contributes to the modulation of neuronal excitation (Chattipakorn and McMahon 2003; Song et al. 2006; Zhang and Thio 2007), the cross-inhibition of A-type gamma-aminobutyric acid ($GABA_A$) receptors (Li and Xu 2002) and short-term plasticity (Zhang et al. 2006). Some studies have indicated that GlyRs are almost absent in the nAc (Sato et al. 1991; Rajendra et al. 1997) and others that the less common and neonatal α_2 subunit indeed is present in the nAc (Racca et al. 1998; Sato et al. 1992; Jonsson et al. 2009), as is, to a lesser extent, the ligand-binding α_1 subunit of the GlyR (Sato et al. 1992; Jonsson et al. 2009).

Ethanol facilitates the function of the generally inhibitory GlyRs, as demonstrated with ion-flux studies in synaptoneurosomes (Engblom and Akerman 1991) and in a variety of other in vitro preparations with electrophysiological techniques (Celentano et al. 1988; Aguayo and Pancetti 1994; Aguayo et al. 1996; Mascia et al. 1996).

4.3 Ethanol and $GABA_A$, 5-HT_3 and NMDA Receptors

The first description of direct ethanol interactions with $GABA_A$ receptors was with receptors in cultured spinal cord neurons, where ethanol was shown to interfere with chloride flux through inhibitory $GABA_A$ receptors (Ticku et al. 1986; Mehta and Ticku 1988). These studies and studies in synaptoneurosomes allowing investigation of chloride flux across neuronal cell membranes prepared from the forebrain revealed that ethanol in relevant concentrations is able to stimulate this flux by itself as well as to potentiate the action of other $GABA_A$ agonists (Suzdak et al. 1986). Patch-clamp studies have illustrated this interaction in much more detail and ethanol is now known to interact differently with different subunit compositions of $GABA_A$ receptors and to induce alterations sin the setups of these subunits in response to chronic ethanol exposure (Ticku 1990; Mhatre and Ticku 1993, 1999; Korpi 1994; Lüddens and Korpi 1995; Hevers and Lüddens 1998). These effects are considered to underlie the alcohol withdrawal syndrome, which is also effectively treated with tapering with cross-tolerant $GABA_A$ agonistic drugs, e.g. benzodiazepines. Lately, focus has been on ethanol's tentative interaction with delta-subunit containing extrasynaptically located $GABA_A$ receptors, which are involved in maintaining tonic inhibition (Olsen et al. 2007; Santhakumar et al. 2007). Interestingly, these receptors are especially sensitive to the partial inverse benzodiazepine receptor agonist Ro 15-4513, which earlier was demonstrated to act as an ethanol antagonist in behavioral experiments (Wallner and Olsen 2008).

In the early 1990s it was discovered that intoxicating concentrations of ethanol potentiate 5-HT_3 mediated ion-currents through a direct interaction with the

receptor, both in a neuroblastoma cell line and in acutely isolated mammalian neurons (Lovinger 1991; Lovinger and White 1991). It was demonstrated that ethanol potentiated the action of 5-HT but did not induce a current by itself. Moreover, with increasing concentrations of 5-HT the potentiating effect of ethanol decreased, thus arguing in favor of a leftward shift of the concentration–response curve, and it was suggested that ethanol stabilizes the receptor in the open channel state (Lovinger and Zhou 1998). More recent studies have in larger detail studied the molecular mechanisms that may be involved in these actions (Zhang et al. 2002; Lopreato et al. 2003; Rüsch et al. 2007).

In contrast to the mainly agonistic actions produced by ethanol in the above-mentioned cys-loop ligand-gated ion channels, ethanol has been demonstrated to be a functional antagonist at the NMDA receptor, a ligand-gated ion channel with a structure different from the above receptors. This receptor, just like the $GABA_A$ receptors, is extremely abundant in the brain as one of the major receptors for glutamate, the brain's most important excitatory neurotransmittor. It was therefore of great interest when in 1989 it was demonstrated both with electrophysiological and ion-flux techniques that ethanol in relevant concentrations (5–50 mM) reduces the amplitude of NMDA-activated currents in hippcampal neurons and NMDA-induced calcium uptake in cerebellar granule cells in primary cultures (Hoffman et al. 1989; Lovinger et al. 1989). The site of the ethanol interaction in these receptors has been debated. Some results have suggested that ethanol interacts with the co-agonistic glycine site on the receptor complex (Rabe and Tabakoff 1990), whereas other investigations have suggested that ethanol is a non-competitive antagonist that does not interfere specifically with any of the known modulatory sites of the receptor (Peoples et al. 1997). Also, the sensitivity to ethanol is similar regardless of whether the receptor contains NR1-1a or NR1-1b subunits with either NR2A or NR2B subunits, indicating that the presence or absence of the N-terminal cassette does not affect the sensitivity of NMDA receptors to ethanol (Popp et al. 1998). Chronic exposure to ethanol both in vitro and in vivo has been demonstrated to increase NMDA receptor numbers and function, as well as to affect receptor subunit expression, effects that may be related to alcohol tolerance and withdrawal phenomena as well as neurotoxicity (Iorio et al. 1992; Davidson et al. 1993; Chandler et al. 1993; Snell et al. 1993, 1996).

5 Ethanol, nAChR and the Mesolimbic Dopamine System

Nicotinic acetylcholine receptors are present both on the cell bodies of the mesolimbic dopamine system and on the neuronal terminals (Jensen et al. 2005). The first studies on a possible involvement of nAChRs in ethanol's dopamine elevating effect were performed in mice, where it was found that the dopamine turnover and locomotor stimulating effects of ethanol were partly blocked by systemic administration of the tertiary nAChR antagonist mecamylamine but not

by the quaternary peripherally acting antagonist hexamethonium, implicating central nAChRs in the effects observed (Blomqvist et al. 1992). Subsequent in vivo microdialysis studies in rats demonstrated that the dopamine release in the nAc and the enhanced dopamine synthesis in the limbic system in response to systemic ethanol were blocked by systemic mecamylamine, supporting a role for central nAChRs in ethanol-induced dopamine enhancement in the nAc (Blomqvist et al. 1993). In an attempt to further locate the nAChRs involved, mecamylamine locally applied in the anterior VTA, but not in the posterior VTA or in the nAc blocked the nAc dopamine elevation after ethanol (Blomqvist et al. 1996; Ericson et al. 2008). Thus, ethanol-induced mesolimbic dopamine activation involves nAChR in the anterior VTA. However, local perfusion of ethanol in the anterior or posterior VTA does not increase dopamine release in the nAc, whereas local perfusion in the nAc does, and this elevation is prevented by nAChR blockade in the anterior VTA (Ericson et al. 2003, 2008; Löf et al. 2007a). Therefore ethanol produces an effect in the nAc that most likely secondarily increases endogenous acetylcholine release and nAChR activation in the anterior VTA. This idea is reinforced by findings that acetylcholine depletion prevents ethanol-induced dopamine release and that ethanol consumption enhances acetylcholine release in the VTA in parallel with dopamine release in the nAc (Ericson et al. 2003; Larsson et al. 2005). The event produced by ethanol in the nAc that eventually leads to nAChR activation in the VTA is probably an interaction with another cys-loop receptor—the GlyR (see below). Ethanol may still interact directly with nAChRs in the VTA, provided that it simultaneously is present in the nAc (Löf et al. 2007a). This could be explained by the fact that ethanol is a co-agonist rather than an agonist at nAChR (Marzalec et al. 1999), and when ethanol is simultaneously applied in the nAc, acetylcholine is released in the VTA, enabling ethanol to influence ventral tegmental nAChRs.

Contrary to the case with nicotine, $\alpha_4\beta_2^*$ nAChRs appear exempt from ethanol's effects outlined above, since di-hydro-β-erythroidine, a specific antagonist at $\alpha_4\beta_2$ nAChRs, does not block ethanol-induced dopamine release (Ericson et al. 2003). Instead $\alpha_3\beta_2$, α_6 and/or β_3 containing nAChRs have been implicated (Larsson et al. 2004; Jerlhag et al. 2006a, b). As regards the α_7 receptors, ethanol is an antagonist rather than agonist at these receptors (Yu et al. 1996; Covernton and Connolly 1997; Aistrup et al. 1999). Consequently, the dopamine-related effects of ethanol are not altered by α_7 receptor blockade. Such blockade also fails to mimic the ethanol effect. In conclusion, the available evidence indicates that ethanol increases mesolimbic dopamine activity via indirect and/or direct activation of $\alpha_3\beta_2$, α_6 and/or β_3 containing nAChRs in the anterior VTA.

Co-administration of ethanol and nicotine produces complex results with respect to ethanol-induced locomotor stimulation in mice, where nicotine either potentiates or counteracts the stimulatory effect of ethanol depending on the doses of both substances. In rats nicotine and ethanol produce additive effects on dopamine release in the nAc, both when the drugs are administered systemically and when ethanol is given systemically and nicotine locally in the VTA (Tizabi et al. 2002, 2007).

Systemic administration of a nicotinic antagonist reduces ethanol consumption in a two-bottle choice test and both ethanol and nicotine self-administration in operant procedures (Blomqvist et al. 1996; Le et al. 2000). Furthermore, local antagonism of nAChRs in the VTA reduces lever-pressing for nicotine as well as ethanol intake and preference in the two-bottle test (Ericson et al. 1998; Le et al. 2000). Dopamine levels in the nAc were concomitantly measured and the results parallel the ethanol consumption findings (Ericson et al. 1998). In analogy with the pharmacological studies, $\alpha_4\beta_2$ receptors mediate nicotine self-administration e.g. (Corrigall et al. 1994) but not alcohol consumption, which rather appears to involve $\alpha_3\beta_2$, α_6 and/or β_3 containing nAChRs (Le et al. 2000; Larsson et al. 2004, Jerlhag et al. 2006a, b). The partial nAChR antagonist varenicline, an established smoking cessation agent, also reduces ethanol consumption in rats (Steensland et al. 2007) and interferes with ethanol's and nicotine's dopamine elevating effects in the nAc (Ericson et al. 2009). However, there are also studies that have failed to demonstrate an ethanol consumption reducing effect of nAChR antagonists (e.g. Dyr et al. 1999).

It has been proposed that nAChRs are involved also in the mediation of ethanol-conditioned dopamine release (Ericson et al. 1998). nAChR blockade may thus prevent a cue-induced dopamine release that prompts the consumer to approach the ethanol bottle. This hypothesis received support when it was shown that a stimulus previously associated with ethanol intake by itself increased dopamine output in the nAc, an effect prevented by nAChR blockade (Löf et al. 2007b). Additionally, responding with conditioned reinforcement for alcohol-associated stimuli involves nAChRs, and $\alpha_3\beta_2$, α_6 and/or β_3 containing receptors, rather than $\alpha_4\beta_2$, may be involved also in these effects (Löf et al. 2007b). Brain acetylcholine systems, probably via nAChR activation, have been implicated in mediating conditioning also to various other rewards (Reid et al. 1998, 1999; Zachariou et al. 2001; Olausson et al. 2004a, b; Brunzell et al. 2006).

Taken together, a certain nAChR population (containing $\alpha_3\beta_2$, α_6 and/or β_3 subunits) in the cell-body region of the mesolimbic dopamine reward system is involved both in ethanol-conditioned activation of the system and in the pharmacological activation produced by ethanol. This coincidence is interesting, since ethanol intake in alcoholics often triggers further ethanol consumption, even though the individual already may be heavily intoxicated. This phenomenon could be explained if ethanol pharmacologically activates the same neuronal mechanisms as those involved in conditioned initiation of ethanol consumption—establishing a *circulus vitiosus*. If the same neurocircuitry mediates conditioned dopamine release to stimuli associated also with other drugs, ethanol's pharmacological activation of this system could contribute to ethanol-induced relapse to other addictive drugs, as often observed among drug dependent individuals.

Also in humans, nAChR blockade decreases the stimulatory and euphoric effects of ethanol (Blomqvist et al. 2002; Chi and de Wit 2003; Young et al. 2005). In addition, some evidence indicates that ethanol consumption as such is decreased by nAChR blockade in alcohol dependent individuals (Petrakis et al. 2008) and recently the nAChR partial agonist varenicline was shown to decrease both alcohol

craving and consumption in an experimental situation in man and in patients undergoing smoking cessation treatment (McKee et al. 2009; Fucito et al. 2011).

6 Ethanol, GlyR and the Mesolimbic Dopamine System

Until quite recently interest concerning GlyR was focused on their role in the spinal cord, whereas they were not believed to serve any major function in the forebrain. However, it has now become clear that the GlyR is present and functionally active also in the forebrain, in e.g. the nAc. Thus, both in situ hybridization, rt-PCR and Western blot experiments have revealed the presence of GlyRs in the nAc, and electrophysiological studies have indicated that there are functional GlyRs both in the nAc and VTA (Ye 2000; Zheng and Johnson 2001). Blockade of these receptors by local perfusion of the GlyR antagonist strychnine in the nAc concentration-dependently and reversibly reduces extracellular dopamine levels, and co-perfusion with glycine concentration-dependently reverses the dopamine reduction induced by strychnine. GlyRs thus are tonically activated and control at least 60% of the dopamine tone in the nAc (Molander and Söderpalm 2005a). Glycine by itself, as well as a glycine uptake inhibitor, increases dopamine levels in some animals but not in others (Molander and Söderpalm 2005a; Lidö et al. 2009). The reason for this is unknown, but could involve a multitude of phenomena, e.g. rapid desensitization of GlyRs in some animals but not in others or differences in receptor setups or subtypes etc. Also the GlyR agonists taurine and β-alanine elevate dopamine levels in the nAc (Ericson et al. 2006, 2010). It has been suggested that activation of inhibitory GlyRs in the nAc decreases the activity of GABAergic neurons projecting backward onto *i.a.* cholinergic afferents in the VTA. A disinhibition of these cholinergic afferents would explain why taurine-induced dopamine release in the nAc is blocked by nAChR antagonism locally in the VTA.

Based on the above findings, GlyRs in the nAc are interesting candidates via which ethanol may increase dopamine levels. Indeed, the dopamine releasing effect of systemic or local (in the nAc) ethanol is blocked by strychnine, but not by the $GABA_A$ channel blocker picrotoxin (see below). Furthermore, and as already mentioned, glycine *per se* enhances dopamine levels in some animals but not in others. However, regardless of whether glycine or a glycine uptake inhibitor increases dopamine levels or not, the dopamine elevating effect of subsequent ethanol administration is prevented (Molander and Söderpalm 2005b; Lidö et al. 2009). It has been suggested that blockade of ethanol-induced dopamine release by strychnine and glycine may derive from receptor blockade and desensitization, respectively. Since the dopamine elevating effect after accumbal ethanol, like that after taurine, is blocked by nAChR antagonism in the VTA, ethanol and taurine probably work through a similar mechanism, i.e. by reducing output from inhibitory backward projecting GABAergic neurons controlling acetylcholine release in the VTA.

The ethanol-GlyR interaction in the nAc has been examined also in ethanol high-preferring rats in a free-choice two-bottle test. In these rats strychnine applied bilaterally in the nAc lowered extracellular dopamine levels, whereas ethanol intake instead increased, but, interestingly, the ingested ethanol failed to elevate accumbal dopamine levels (Molander et al. 2005). Glycine, on the other hand, again increased dopamine levels in some but not all animals and ethanol preference was significantly reduced only in rats that responded to glycine with a dopamine elevation (glycine responders). The amount of ethanol consumed by the glycine responding group failed, however, to further increase dopamine levels (Molander et al. 2005). In these experiments the reduced ethanol intake after glycine may be due to a substitution phenomenon, whereas the increased intake after strychnine could be due to blockade of the ethanol effect resulting in an attempt to compensate for this.

In the above experiments, which applied a counter-balanced two-day design, there was a clear and highly significant difference between the glycine responders and the glycine nonresponders also with respect to how they responded to Ringer, i.e. the control solution. The responders consumed only slightly and nonsignificantly more ethanol than the nonresponders but the dopamine elevation after ethanol was approximately 100% larger (Molander et al. 2005). It was also noted that the frequency of glycine responders was approximately 50% among the ethanol high-preferring animals, compared to approximately 25% among ethanol naïve animals (Molander and Söderpalm 2005a). These results indicate that glycine responders are more sensitive to the dopamine elevating effect of ethanol and that this feature may promote a high preference for ethanol or that a high ethanol intake produces glycine responders.

In this context it is interesting to note that systemic administration of a glycine uptake inhibitor, which raises extracellular glycine levels in the nAc by approximately 80% has been demonstrated to dramatically reduce ethanol intake in two different laboratories without any signs of tolerance development (Molander et al. 2007; Vengeliene et al. 2010). Tolerance is commonly observed in rats after ethanol intake modulating compounds, e.g. acamprosate, naloxone, selective serotonin reuptake inhibitors and 5-HT$_{1A}$ receptor agonists (Hedlund and Wahlström 1998a, b), and it is possible that this is part of the reason why these drugs show a limited efficacy in man.

As alluded to earlier, local administration of taurine in the nAc increases dopamine levels in a similar manner as ethanol; the effect is blocked by strychnine in the nAc, by the nAChR antagonist mecamylamine in the VTA and by systemic administration of vesamicol (an acetylcholine depleter) (Ericson et al. 2006). This together with the fact that systemic administration of ethanol increases extracellular levels of taurine in nAc (Dahchour et al. 1996) point toward taurine as a possible mediator of ethanol's dopamine elevating effect. Indeed, if ethanol is systemically administered in a hypertonic NaCl solution (3.6%) instead of in a physiological one (0.9%) both the taurine *and* the dopamine elevation induced by ethanol is completely blocked. However, if a low, by itself inactive concentration of taurine is concomitantly perfused in the nAc the dopamine elevation is rescued

(Ericson et al. 2011). These findings indicate that ethanol and the ethanol-induced taurine elevation interact, probably in the GlyR, to produce dopamine release. Furthermore, the increased taurine levels observed after ethanol could derive from osmotic alterations, since taurine is an osmoregulator and since ethanol induces astrocyte swelling in cultures. It was recently demonstrated that a pharmacological manipulation (furosemide) that blocks astrocyte swelling also reduces baseline taurine levels and prevents ethanol from elevating both taurine and dopamine, when applied in the nAc (Adermark et al. 2011).

7 Ethanol, the Mesolimbic Dopamine System and 5-HT$_3$ Receptors

5-HT$_3$ receptors are present in the nAc and a few studies have demonstrated that their activation facilitates accumbal dopamine release, although they do not appear to be tonically activated by serotonin (Jiang et al. 1990; Chen et al. 1991; Parsons and Justice 1993). Several reports using different methodologies, such as in vivo voltammetry and in vivo microdialysis, have shown that the increased dopamine output produced by ethanol in the nAc can be prevented by local or systemic administration of drugs antagonizing brain 5-HT$_3$ receptors (Carboni et al. 1989; Wozniak et al. 1990; Yoshimoto et al. 1992, 1996; Campbell and McBride 1995). Since it is known that 5-HT$_3$ receptors are located on the terminals of dopaminergic neurons and since ethanol interacts directly with 5-HT$_3$ receptors (see above), it is plausible that the effect observed is due to blockade of ethanol's direct interaction with these. However, it is still unclear whether this 5-HT$_3$ mediated effect in the nAc also involves nAChRs in the VTA, since antagonism of these latter receptors apparently blocks the dopamine releasing effect of systemic administration of ethanol. One possibility is that the 5-HT$_3$ active drugs used in the experiments are not selective for 5-HT$_3$ receptors but interfere also with other ligand-gated ion channels, e.g. GlyRs. Indeed, there are reports suggesting this (Chesnoy-Marchais 1996; Chesnoy-Marchais et al. 2000). In that case the sequence of events could be the same as discussed earlier with respect to ethanol interference with GlyRs in the nAc.

A series of studies have suggested that 5-HT$_3$ receptors also in the VTA are involved in dopamine activation and positive reinforcement (Liu et al. 2006; Rodd et al. 2007). These investigators also claim that ethanol produces its dopamine activating and reinforcing effects via an interference with 5-HT$_3$ receptors in the posterior, but not the anterior, VTA (Rodd et al. 2005, 2010). It should be noted, however, that in some of these experiments a paradigm using local self-injections of ethanol in the posterior VTA has been applied and it is therefore still not entirely clear whether these mechanisms are involved also in the dopamine elevating and reinforcing effects of ethanol observed after systemic administration, e.g. after oral self-administration.

8 Ethanol, the Mesolimbic Dopamine System and $GABA_A$ Receptors

$GABA_A$ receptors are abundant along the mesolimbic dopamine system, and interference with these either in the VTA or in the nAc will influence dopamine output in the nAc. Indeed when the $GABA_A$ channel antagonist picrotoxin is perfused locally in the nAc the extracellular dopamine levels increase. Thus $GABA_A$ receptors in this area appear to tonically reduce dopamine output. In consonance with these findings it has been demonstrated that local administration of $GABA_A$ agonists reduces extracellular dopamine levels in the nAc (Zetterström and Fillenz 1990; Tanganelli et al. 1994; Ferraro et al. 1996; Löf et al. 2007b). However, it has recently been suggested that positive $GABA_A$ modulators may release dopamine in the nAc via disinhibition of dopaminergic neurons in the VTA (Tan et al. 2010). This effect is suggested to derive from $GABA_A$ mediated inhibition of GABAergic interneurons in the VTA. It is possible that a balance with respect to how $GABA_A$ receptors are activated on dopamine terminals in the nAc and in the VTA, respectively, will determine the net outcome after different $GABA_A$ agonists after acute and chronic treatments.

With respect to ethanol it has been suggested that also this drug would disinhibit VTA dopamine neurons via stimulation of $GABA_A$ receptors located on inhibitory interneurons. The actual experimental evidence for this is, however, lacking. On the contrary recent evidence suggests that $GABA_A$ stimulation induced by ethanol in the nAc balances its stimulatory effect and that at later stages after ethanol administration this $GABA_A$ stimulation in fact dominates and therefore reduces the stimulatory response and may even suppress dopamine levels below baseline levels. This conclusion was reached after applying the $GABA_A$ channel blocking agent picrotoxin locally in the nAc while simultaneously perfusing ethanol in the same region (Löf et al. 2007b).

9 Ethanol, the Mesolimbic Dopamine System and Glutamate Receptors

The relationship between the glutamate system and mesolimbic dopamine activity appears complicated. There are glutamatergic projections both from the prefrontal cortex and from deeper brain regions, e.g. the amygdala, hippocampus and the lateral hypothalamus, to both the VTA and the nAc, and various glutamatergic receptors, both ionotropic and metabotropic, are present in these areas. A number of studies using various in vivo techniques have been performed to establish whether glutamatergic receptors control mesolimbic dopamine activity but the results are inconsistent (Blaha et al. 1997; Floresco et al. 1998; Howland et al. 2002; Youngren et al. 1993; Moghaddam et al. 1990). Even though some studies show that stimulation of ionotropic glutamate receptors in the nAc shell increases

extracellular dopamine levels in the same area others have failed to observe such effects or shown the opposite. The results may be more consistent with respect to interactions in the VTA, where NMDA receptors may be tonically activated and contribute to maintain basal dopamine levels, and where additional NMDA receptor stimulation increases dopamine release (Karreman and Moghaddam 1996; Karreman et al. 1996).

Depending on dose, ethanol may increase or decrease extracellular glutamate levels in the nAc (Moghaddam and Bolinao 1994), but there are no in vivo studies demonstrating that ethanol-induced accumbal dopamine release can be prevented by glutamate receptor antagonists applied in this area. Furthermore, with respect to the ionotropic NMDA receptors in the VTA, these would probably be blocked by ethanol, since ethanol has NMDA antagonistic properties (see above). An ethanol-induced blockade of these receptors would be expected to reduce rather than to increase dopamine release, and a reduction of dopamine release is clearly not observed after systemic ethanol. However, it cannot be excluded that ethanol-induced blockade of these receptors contributes to mitigate ethanol's stimulatory action on mesolimbic dopamine neurons produced via other mechanisms.

In vivo evidence for ethanol-induced direct or indirect interference with NMDA receptors or other glutamate receptors being involved in ethanol-induced dopamine activation is hence in essence lacking. However, using two different in vitro preparations it was recently demonstrated that AMPA receptor function on midbrain dopamine neurons was enhanced by ethanol administration and that this effect might be due to an interference with dopamine D1 receptors on glutamatergic terminals, which in turn facilitates glutamate release and AMPA activation leading to somatodendritically released dopamine, further dopamine D1 activation etc. (Deng et al. 2009; Xiao et al. 2009). Interestingly, in vivo microdialysis studies have suggested a scenario involving glutamate release also in nicotine-induced dopamine activation (Schilström et al. 2000, 2003), further underlying mechanistic overlaps between ethanol and nicotine (see above). But, again, whether this interaction in the case of ethanol is present also in vivo remains to be determined.

10 Ethanol, the Mesolimbic Dopamine System and Opioid Receptors

The beneficial effect of opioid antagonists on excessive alcohol consumption has been a subject of interest within the research society for a long time (Altshuler et al. 1980). Transmission of the endogenous opioid system is highly present within the brain reward system and participates in the modulation of reward circuits (Mansour et al. 1995; Trigo et al. 2010). Modulation of opioid receptors, using μ- and β-receptor antagonists as well as β-endorphin knockout mouse models, was found to alter the ethanol-induced dopamine elevation (Acquas et al. 1993; Benjamin et al. 1993; Gonzales and Weiss 1998) and reduce ethanol intake

(for review see Trigo et al. 2010). However, the exact underlying mechanism for the involvement of opioid receptors in the dopamine elevating properties of ethanol remains to be established.

Acute ethanol exposure increases brain enkephalin (Seizinger et al. 1983) and β-endorphin (Schulz et al. 1980) content, and a correlation has been observed between increased β-endorphin level and the risk of alcoholism in humans (Gianoulakis et al. 1996). Chronic ethanol exposure leads to alterations in the opioid system, which is suggested to participate in the development of alcohol addiction (Gianoulakis 1996). The different types of opioid receptors appear to be involved in mediating ethanol consumption by separate mechanisms. Administration of the non-specific opioid antagonists naloxone and naltrexone is known to reduce voluntary ethanol intake in various models and, in addition, a specific δ-receptor antagonist was displayed to have the same effect (Frohlich et al. 1994), as did a specific μ-receptor antagonist (Hyytiä 1993).

There may be several points within the system where opioid receptors can influence ethanol-induced dopamine elevation. For example, in the VTA, both ethanol and endogenous opioids modulate GABAergic neurotransmission via μ-receptors, which could indirectly influence dopaminergic transmission (Xiao and Ye 2008; Xiao et al. 2007; Trigo et al. 2010). A decreased GABAergic influence enables other transmitters, such as acetylcholine or glutamate, to activate dopamine neurons with the result of increased nAc dopamine output (see Spanagel and Weiss 1999 or Trigo et al. 2010 for review).

11 Ethanol, the Mesolimbic Dopamine System and Ghrelin

Ghrelin, a gastric peptide important in regulating hunger and appetite, has in a recent line of studies been implicated to be of importance for the reinforcing properties of alcohol. Human studies found that plasma levels of ghrelin are higher in abstinent alcoholic individuals as compared to controls (Kim et al. 2005; Kraus et al. 2005), whereas acute alcohol consumption suppresses plasma levels of the hormone (Calissendorff et al. 2005). In addition the elevated levels of ghrelin in alcoholics correlated with craving (Addolorato et al. 2006) implicating an influence on the brain reward system.

Ghrelin was demonstrated to be the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Wren et al. 2000), a G-protein-coupled receptor involved in secretion of growth hormone, as well as prolactin and ACTH. Furthermore, this receptor is expressed in various brain regions including areas within the brain reward system (Guan et al. 1997) where it could influence brain reward signaling. Indeed, preclinical studies using rodents demonstrated ghrelin to increase dopamine overflow in the nAc by means of in vivo microdialysis as well as to increase locomotor activity, by a mechanism involving nAChRs (Jerlhag et al. 2006a, 2006b). Subsequent studies identified GHS-R located in both the VTA and in the laterodorsal tegmental area to mediate the effects of ghrelin on accumbal

dopamine levels (Jerlhag et al. 2007) as well as alpha-conotoxin MII sensitive nAChRs (targeting $\alpha_3\beta_2$, β_3 and/or α_6 receptor subunits) (Jerlhag et al. 2008) thus displaying an activation of the mesolimbic dopamine system similar to that of ethanol (Söderpalm et al. 2000, 2009).

Investigating a possible interaction between ghrelin signaling and alcohol in the mesolimbic dopamine system, ghrelin administration into the ventricles, the VTA or into the laterodorsal tegmental nucleus increased alcohol intake in mice (Jerlhag et al. 2009) whereas administration of ghrelin into the lateral hypothalamus, the paraventricular nucleus or into the nAc left the voluntary alcohol intake in rats unaltered (Schneider et al. 2007). This would thus indicate that GHS-R located in the cellbody region of the mesolimbic dopamine system or on cholinergic afferents projecting to the VTA are of functional importance for alcohol intake in rodents. In line with this, administration of a GHS-R1A antagonist decreased voluntary ethanol intake in mice (Jerlhag et al. 2009; Kaur and Ryabinin 2010) as well as in rats (Landgren et al. 2011). Supporting these functional studies administration of a GHS-R1A antagonist or using a GHS-R1A knockout mice prevented ethanol-induced elevation of locomotor activity, nAc dopamine release and conditioned place preference (Jerlhag et al. 2009). Taken together, pre-clinical findings suggest that administration of a GHS-R1A antagonist could be a potential new pharmacotherapy for alcoholism.

12 Alcohol Relapse-Preventing Drugs and the Mesolimbic Dopamine System

The oldest pharmacological treatment for alcoholism, disulfiram, inhibits aldehyde dehydrogenase, thus producing aversive side effects of alcohol consumption due to accumulation of acetaldehyde. However, even though this is probably the main action of the drug, disulfiram may also influence the mesolimbic dopamine activity, since it decreases noradrenaline and increases dopamine levels due to inhibition of dopamine-β-hydroxylase (Karamanakos et al. 2001; Bourdélat-Parks et al. 2005). Moreover, inhibition of acetaldehyde dehydrogenase secondarily affects dopamine also via accumulation of tetrahydropapaverin (Yao et al. 2010). Supporting this hypothesis disulfiram was recently demonstrated to block the development of behavioral sensitization to the stimulant effects of ethanol in mice (Kim and Souza-Formigoni 2010).

Besides disulfiram there are currently another two well-established pharmacotherapies for alcoholism, naltrexone and acamprosate. The unselective opioid antagonist naltrexone was previously mentioned. Naltrexone decreases voluntary ethanol consumption in rodents and prevents ethanol from producing a dopamine elevation (Volpicelli et al. 1986, see Trigo et al. 2010 for review). Interestingly, human studies have revealed a functional variation of μ-opioid genes, where a certain variant results in increased subjective alcohol responses (Ray and Hutchison 2007). This variant may thus contribute to explaining the great

variability in response to alcohol and may also confer sensitivity to treatment with naltrexone (Oslin et al. 2003). Furthermore, Ramchandani et al. (2011) recently demonstrated both in man and transgenic mice that presence of this receptor polymorphism is associated with dopamine release after alcohol administration.

On its way into clinical practice is another ligand for the opioid receptor, nalmefene, which is similar to naltrexone in being an antagonist at the μ-opioid receptor. However, nalmefene also demonstrates affinity for the κ-opioid receptor making the antagonistic profile somewhat different (Michel et al. 1985). Nalmefene was demonstrated to decrease ethanol intake more than naltrexone in a comparative study in rats (Walker and Koob 2008). Further studies demonstrated intra accumbens administration of nalmefene to be very potent in decreasing ethanol intake in ethanol preferring rats emphasizing the importance of the opioid system in regulating ethanol intake (Nealey et al. 2011).

Another pharmacotherapy targeting the brain reward system is acamprosate. This alcohol relapse-preventing drug has the most documented mechanism involving the mesolimbic dopamine system of all three alcohol relapse-preventing compounds. The mechanisms underlying its ethanol-intake reducing effects have been suggested to include a wide range of receptors in the mesolimbic dopamine system such as $GABA_A$, NMDA, metabotropic glutamate receptors and GlyRs (Boismare et al. 1984; Zeise et al. 1993; Dahchour et al. 1998; Harris et al. 2002). Acamprosate has been demonstrated to increase nAc dopamine by itself and has also been found to prevent ethanol-induced elevation of dopamine in the terminal region (Olive et al. 2002; Chau et al. 2010a). Even though the compound often is suggested to normalize a hyperglutamatergic state, disclosed after excessive alcohol intake, other studies have pointed in different directions. It was recently shown that the dopamine elevation produced by acamprosate is prevented by antagonism of either GlyRs in the nAc or nAChRs in the VTA, much in line with the case of alcohol (Chau et al. 2010a). Furthermore, in a voluntary ethanol consumption paradigm rats decreased their ethanol intake after systemic administration of acamprosate. This effect was reversed by microinjections of the GlyR antagonist strychnine locally in the nAc (Chau et al. 2010b), thus demonstrating that nAc GlyRs are of functional importance for mediating the decrease in voluntary ethanol intake produced by acamprosate.

In conclusion, there is experimental support for the notion that all currently available pharmacological treatment options for alcohol use disorders interact with the mesolimbic dopamine system, and, in addition, some evidence that this interaction may be involved in the functional effects observed.

13 Summary and Implications

Both animal and human studies demonstrate that ethanol releases dopamine in the ventral striatum, an essential component of the brain reward system. This dopamine release is likely involved in the initial positive reinforcing effects of ethanol

and at later stages probably both in the positive and negative reinforcing effects of the drug. The latter may be inferred from findings in rats that ethanol's dopamine elevating effect is preserved after subchronic and chronic administration despite the lowered dopamine baseline functioning at this stage (Diana et al. 1993), that in itself may reflect anhedonia (Koob and LeMoal 2001). In fact, the relative dopamine elevation appears larger when dopamine baseline levels have dropped and reaches the same absolute level as after acute ethanol administration. These observations fit with the clinical experience that alcoholics not only are "normalized" by drinking ethanol but also still may become stimulated and euphoric after ethanol intake. Drinking behavior is therefore most likely driven both by negative and positive reinforcement in the alcohol dependent individual.

As outlined above numerous mechanisms have been identified that appear to be involved in mediating ethanol-induced dopamine activation. A major challenge for future research will be to relate these mechanisms to each other, e.g. whether the GlyR involvement is up- or down-stream to e.g. the μ-opioid receptor or whether they act in parallel. Also, there is a shortage of studies in chronically ethanol-exposed animals, i.e. whether the mechanisms so far identified are involved also at later stages. An indication that this may be the case is that the absolute dopamine elevation produced by ethanol after subchronic ethanol administration appears *exactly* the same as after acute administration to naïve animals. This finding may seem odd but is in fact perfectly compatible with the suggestion that ethanol's major interaction with the mesolimbic dopamine system is to lift a major break on the system. Such a view would indicate that the activity of the break is enhanced after subchronic ethanol—this would explain the reduced baseline dopamine activity and the relatively enhanced dopamine release when the break is lifted at this stage, and that the absolute dopamine levels do not exceed what was observed at the starting point—i.e. ethanol can do no more than lifting the break(!).

Already some experiments have been performed in order to examine the relationship between GlyR and nAChR in the mesolimbic dopamine activating effect of ethanol. The model proposed based on these findings is presented in Fig. 1 and now awaits experiments aimed at incorporating also the other major players, e.g. the my-opioid receptors, 5-HT$_3$ receptors and NMDA receptors. The model already at this stage has some important implications.

First, involvement of GlyRs located on target neurons in the nAc in the reinforcing effects of ethanol is theoretically interesting. Thus a situation analogous to that of opiates may be at hand—opiates both enhance dopamine release and directly via inhibitory μ-receptors reduce target neuronal activity in the nAc. This has been advanced as a possible explanation to why destruction of dopamine neurons does not abolish opiate self-administration (Pettit et al. 1984). Dopamine lesions generally fail to reduce also ethanol self-administration (see Introduction). According to the above the explanation may be that ethanol besides releasing dopamine also directly, via GlyRs, inhibits neuronal activity in the nAc (that otherwise would also have been inhibited via dopamine D$_2$ receptors). Moreover, if the reward value is determined by to what extent the target neurons in the nAc are inhibited rather than by how much dopamine is liberated, this could explain

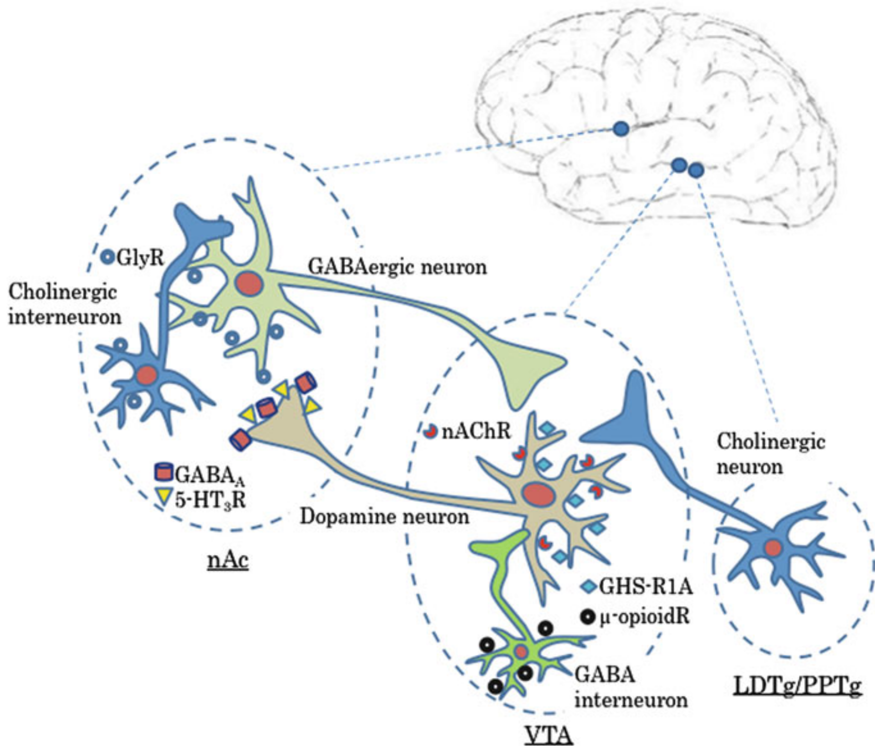

Fig. 1 Simplified schematic figure of a selection of the important participators mediating ethanol-induced activation of the mesolimbic dopamine system (nAc = nucleus accumbens, VTA = ventral tegmental area, LDTg = laterodorsal tegmental nucleus, PPTg = pedunculopentine nucleus, GlyR = glycine receptor, nAChR = nicotinergic acetylcholine receptor, $GABA_A$ = GABA receptor type A, 5-HT$_3$R = Serotonin receptor type 3, μ-opioidR = Opioid receptor of μ-type, GHS-R1A = Ghrelin receptor type 1A). Ethanol may primarily act in the nAc, where it influences GlyRs, which, in turn, decreases the inhibitory tone mediated by GABAergic neurons projecting to the VTA. Thus, acetylcholine is released into the VTA, where it activates dopaminergic neurons via specific nAChRs resulting in elevation of dopamine. Other entry points into this regulatory circuit by ethanol may be via μ-opioidR, GHS-R1A or possibly via 5HT$_3$R

why ethanol, despite releasing relatively small amounts of dopamine, has a high reward value. Thus ethanol, like opiates, may be able to by-pass the dopamine system in the brain reward system.

Second, the findings that the same subtypes of nAChRs appear to be involved in ethanol-induced mesolimbic dopamine activation and in ethanol-conditioned activation of the dopamine system may implicate the same neuronal circuitry in both actions. In that case anticipation of ethanol reward should increase extra-cellular levels of any of the endogenous GlyR ligands in the nAc. Interestingly, a recent report indicates that this may indeed be the case—anticipation of ethanol intake raises extracellular glycine levels in the nAc (Li et al. 2008). The question arises as to whether this is a pathway for ethanol-conditioned activation of the

mesolimbic dopamine system only or whether it pertains also for conditioning to other rewards. A well-known "clinical" observation is that ethanol intake triggers "relapse" to a multitude of consummatory behaviors, e.g. intake of illicit drugs, smoking, sex, gambling, aggression and food intake. These effects have often been ascribed a general disinhibitory action of ethanol but could also involve ethanol-induced activation of a neurocircuitry of relevance for reward conditioning in general.

Third, if ethanol produces its dopamine elevating effect by interfering with backward projecting breaks to the VTA, involving i.a. GlyRs in the nAc and nAChRs in the VTA, it may be hypothesized that chronic ethanol exposure produces an adaptation of this system by a down-regulation of GlyR and/or nAChR function and/or of other components in this chain of events. Such a down-regulation is likely to result in decreased basal dopamine activity, especially as it has been observed that the same GlyR population in the nAc sustains basal accumbal dopamine levels. Indeed, and as stated above, during withdrawal from chronic ethanol treatment extracellular dopamine levels are profoundly reduced in the nAc (Diana et al. 1993) and this biochemical alteration has been associated with a demand for higher self-stimulation thresholds in an animal model of hedonia/anhedonia (Schulteis et al. 1995). Similar baseline dopamine reductions after chronic cocaine self-administration have been shown to correlate with enhanced drug self-administration in dependent animals, indicating that this neurochemical effect drives drug intake (Ahmed and Koob 2005). It will now become of importance to pin-point the alterations of the neurocircuitry proposed above accounting for the reduced dopamine baseline levels, as a reversal of these theoretically would reduce alcohol craving and intake.

Lastly, it appears that the current alcohol relapse-preventing drugs in various ways interfere with the mesolimbic dopamine system. This is interesting and strengthens the case for this system as important for modulating alcohol craving and consumption. However, it could be argued that the limited effect sizes of these compounds would be discouraging for finding really efficient drugs targeting this system. The more recent discoveries of targets related more to the core of ethanol's action along this system could possibly open up for more efficient drugs, that could rest on either substitution and/or antagonism principles for combating alcohol use disorders. Ongoing clinical trials with nAChR and GlyR modulators will reveal whether this is a rewarding strategy.

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What is in that Drink: The Biological Actions of Ethanol, Acetaldehyde, and Salsolinol

Gerald A. Deehan Jr., Mark S. Brodie and Zachary A. Rodd

Abstract Alcohol abuse and alcoholism represent substantial problems that affect a large portion of individuals throughout the world. Extensive research continues to be conducted in an effort to identify the biological basis of the reinforcing properties of alcohol in order to develop effective pharmacotherapeutic and behavioral interventions. One theory that has developed within the alcohol field over the past four decades postulates that the reinforcing properties of alcohol are due to the action of the metabolites/products of alcohol within the central nervous system (CNS). The most extreme version of this theory suggests that the biologically active metabolites/products of alcohol, created from the breakdown from alcohol, are the ultimate source of the reinforcing properties of alcohol. The contrary theory proposes that the reinforcing properties of alcohol are mediated completely through the interaction of the ethanol molecule with several neurochemical systems within the CNS. While there are scientific findings that offer support for both of these stances, the reinforcing properties of alcohol are most likely generated through a complex series of peripheral and central effects of both alcohol and its metabolites. Nonetheless, the development of a greater understanding for how the metabolites/products of alcohol contribute to the reinforcing properties of alcohol is an important factor in the development of efficacious pharmacotherapies for alcohol abuse and alcoholism. This chapter is intended to provide a historical perspective of the role of acetaldehyde (the first metabolite of

G. A. Deehan Jr. (✉) · Z. A. Rodd
Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202-4887, USA
e-mail: gdeehan@iupui.edu

M. S. Brodie
Department of Physiology and Biophysics, University of Illinois, Chicago, IL, USA

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alcohol) in alcohol reinforcement as well as review the basic research literature on the effects of acetaldehyde (and acetaldehyde metabolites/products) within the CNS and how these function with regard to alcohol reward.

Keywords Acetaldehdye · Alcohol · Alcoholism · Alcohol metabolite · Alcohol · Aldehyde dehydrogenase reinforcement · Antabuse · Disulfiram · Salsolinol · Tetrahydrobetacarbolines · Tetrahydroisoquinoline alkaloids

Abbreviations

Triazole	3-amino-1,2,4-triazole
ACD	Acetaldehyde
ADH	Alcohol dehydrogenase
I_A	A-type potassium current
BEC	Blood ethanol concentration
CNS	Central nervous system
CPP	Conditioned place preference
DOR	Delta opioid receptor
2 D_2	Dopamine
DA	Dopamine
EtOH	Ethanol
GABA	Gamma-aminobutyric acid
GLU	Glutamate
I_H	Hyperpolarization-activated inward current
ICV	Intra-cerebral ventricular
IV	Intra-venous
mPFC	Medial prefrontal cortex
μM	Micro-molar
MOR	Mu opioid receptor
NIAAA	National Institute for Alcohol Abuse and Alcoholism
AcbC	Nucleus accumbens core
AcbSh	Nucleus accumbens shell
Acb	Nucleus accumbens
$5HT_3$	Serotonin 3
5-HT	Serotonin
TBCs	Tetrahydrobetacarbolines
SAL	Salsolinol
THIQs	Tetrahydroisoquinoline alkaloids
THP	Tetrahydropapaveroline
ALDH	Aldehyde dehydrogenase
US FDA	United States Food and Drug Administration
VTA	Ventral tegmental area
WHO	World Health Organization

Contents

1 Introduction 165
1.1 Relevance and History of Acetaldehyde and Alcoholism 167
1.2 Acetaldehyde Production in CNS 169
2 Acetaldehyde Reactivity 169
2.1 Alcohol, Acetaldehyde, and Acetaldehyde Products 170
3 Acetaldehyde is Pharmacologically Active in the CNS 171
3.1 Acetaldehyde in the VTA: In Vivo Electrophysiology 172
3.2 Acetaldehyde in the VTA: In Vitro Studies 173
3.3 Differences in the Action of Acetaldehyde: Methodological Considerations 173
3.4 Differences Between Acetaldehyde and Alcohol: Electrophysiological Studies ... 174
3.5 Behavioral Pharmacology of Acetaldehyde Within the Mesolimbic Dopamine System 175
4 General Summary 177
References 178

1 Introduction

The vast majority of North Americans and Europeans consume alcoholic beverages during their lives. The imbuement of alcoholic beverages is associated with the goal of obtaining a positive emotional state. The biological basis of the reinforcing properties of alcohol has not been completely established. A recurring theory in the alcohol field is that the reinforcing properties of alcohol are not produced by the ethanol (EtOH) molecule itself, but are dependent upon the action of EtOH metabolites/products. This stance asserts that EtOH is a pro-drug; EtOH is the base compound for biologically active metabolites/products that are the ultimate source of the reinforcing properties of EtOH. Therefore, to understand the biological basis of alcoholism, there is a need to study the metabolites/products of EtOH. Figure 1 is a general schematic that represents the main aspects of the central and peripheral metabolism of alcohol, and the pharmacological interactions of each metabolite/condensation product, elucidated by research over the past century.

The principles underlying the ‘EtOH is a pro-drug’ theory are; (1) the concentrations required to observe EtOH effects in the CNS are too high for conventional pharmacological processes, (2) various behavioral/physiological consequences of alcohol consumption are observed for durations which surpass the bioavailability of EtOH within the system, and (3) metabolism of EtOH to acetaldehyde (ACD) within the CNS mediates most, if not all, of the CNS effects of EtOH. The contrary hypothesis holds that EtOH directly interacts with a number of neurotransmitter systems and it is through these interactions that alcohol exerts its effects. The ‘EtOH Alone’ hypothesis asserts that ACD is aversive in the periphery and exists for such a short time period that it could not possibly mediate the persistent effects observed following alcohol intoxication.

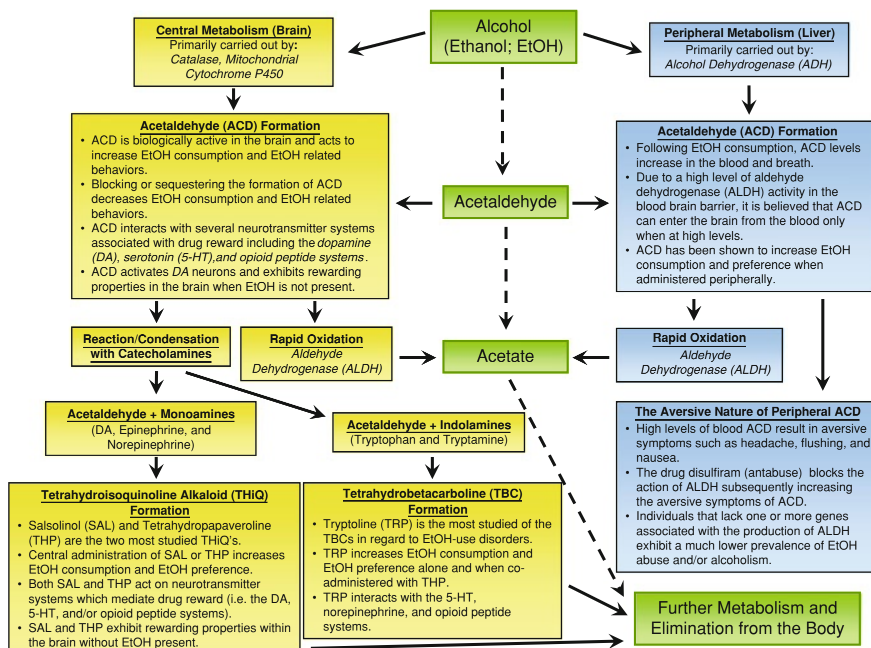

Fig. 1 General schematic representation of the central and peripheral metabolic pathways that function to eliminate alcohol from the human body

Regardless of these opposing stances, genetic studies have clearly linked ACD metabolism with a risk for alcoholism. Specifically, genetic polymorphisms in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) reduce the likelihood of the development of alcoholism (Edenberg 2011). Such polymorphisms are in genes that encode proteins that regulate the production and metabolism of alcohol and ACD, with both types of polymorphisms ultimately affecting ACD levels in the body. Peripheral increases in ACD levels produce an aversive state (e.g., flush, nausea, and sweating) which reduces the likelihood of continued EtOH consumption (Peng and Yin 2009). Recent research has established a polygenic contribution of the ADH gene cluster outlining a likely role for several ADH genes in the development of alcoholism (Frank et al. 2012). Alterations in the ALDH2 gene, and the subsequent increase in ACD following EtOH consumption, have been thoroughly documented as protective in nature, with regard to the development of alcoholism amongst Asian populations (Ball 2008). Emerging evidence suggests that such protection may occur through two mechanisms: (1) an increase in the aversive effects of EtOH through increased ACD levels in the periphery and (2) a decrease in EtOH reward through a functional alteration in dopamine (DA) metabolism (Lu et al. 2010). Inhibition of the ALDH2 gene acted to increase brain levels of tetrahydropapaveroline (THP; a tetrahydroisoquinoline) effectively decreasing cocaine-induced DA signaling within the reward pathway of the brain (Yao et al. 2010) and it has been documented that

higher levels of THP decrease EtOH consumption and preference (Duncan and Deitrich 1980). Thus, genetic polymorphisms may exhibit protective properties through a number of peripheral and central mechanisms working in tandem.

Currently, there are three pharmacotherapeutic agents for the treatment of alcoholism that are approved by the United States Food and Drug Administration (US FDA). These compounds have limited efficacy in preventing relapse to alcohol consumption or reducing on-going alcohol consumption (Bergmark 2008). Despite the decades of research, the biological basis of the effects of alcohol is not unequivocally known. The failure to develop efficacious pharmaceuticals for the treatment of alcoholism may be predicated upon the failure to fully determine the complex biological consequences of EtOH consumption, and that increasing the understanding of the role of ACD and ACD products in the reinforcing properties of EtOH may assist in the development of potential future pharmacotherapies for the treatment of alcoholism. The intent of this review is to provide a historical perspective of ACD and review the basic research literature of the effects of ACD (and ACD metabolites) within the CNS.

1.1 Relevance and History of Acetaldehyde and Alcoholism

Alcoholism represents a substantial health issue in the United States and throughout the world. Currently, there are over 18 million Americans that abuse alcohol or are classified as alcohol-dependent (NIAAA 2008). The World Health Organization has estimated that 140 million individuals throughout the world could be currently diagnosed with alcohol dependency (WHO 2003). For perspective, 140 million is approximately the population of Russia, the nineth largest country in the world by population. In the United States and Eastern Europe, between 10 and 20% of males, and 5–10% of females, could be diagnosed with alcohol dependency (WHO 2003).

Like the majority of drugs of abuse, the reinforcing action of alcohol primarily occurs within the DA reward pathway, which originates in the ventral midbrain (ventral tegmental area; VTA) and projects to forebrain regions that include the nucleus accumbens (Acb) and the medial prefrontal cortex (mPFC) (Koob et al. 1998; McBride et al. 1999). Furthermore, manipulation of the DA, serotonin (5-HT), Gamma-aminobutyric acid (GABA), opioid, and glutamate (GLU) systems within the DA reward pathway, or within structures or areas that project to the DA reward pathway, has been found to effectively alter alcohol-related behaviors in preclinical research (Vengeliene et al. 2008; Koob et al. 1998).

Over the past century, researchers have described the aversive effects of alcohol following the ingestion of a number of compounds which, in turn, significantly affected alcohol-related behaviors in both preclinical as well as clinical populations. Koelsch (1914) described a number of transitory symptoms following alcohol consumption in factory workers manufacturing calcium cyanamide, an organic compound originally synthesized for the production of metals. Following

even a minimal intake of alcohol, individuals exposed to calcium cyanamide would become “hypersensitive to alcohol” and experience flushing (redness) of the face, headaches, shortness of breath, and increased heart rate eventually followed by fatigue (Koelsch 1914). Chifflot (1916) reported that consumption of an ink cap mushroom (coprinus atramentarius) prior to consuming alcohol displayed a similar ‘aversive’ reaction to EtOH consumption. Coprine, an amino acid in the ink cap mushroom, was determined to produce the aversive physical responses to alcohol consumption (Reynolds and Lowe 1965). Another naturalist observation reported that workers at a rubber parts plant exposed to tetramethylthiuram experienced the similar redness in the face, increased heart rate, and palpitations when consuming alcohol (Williams 1937). This led Williams to postulate: “If the chemical compound is not harmful to man, one wonders whether one had discovered the cure for alcoholism.” During a research attempting to treat intestinal worms, Erik Jacobsen and Jens Hald self-treated themselves with tetraethylthiuramdisulphide, and both men reported similar aversive symptoms (redness of the face, increased heart rate, sleepiness, etc.) following alcohol consumption. Additionally, Hald and Jacobsen (1948) subsequently indicated that blocking ALDH) resulted in a sharp increase in blood levels of ACD which in turn resulted in an increase in the aversive side effects of drinking (i.e., flushing of the skin, headaches, nausea, and shortness of breath). Tetraethylthiuramdisulphide has since been given the name disulfuram (marketed as antabuse) and was the first compound approved for the treatment of alcoholism by the US FDA.

While the exact physiological actions of the aforementioned compounds were not immediately clear, further research identified that they had a common mechanism of action in that they blocked the action of the enzyme ALDH which acts to break down ACD, the first metabolite of alcohol. Early research identified a direct relationship between alcohol consumption and blood ACD levels as Stotz (1943) reported that binge drinking episode (BEC 100–180 mg%) produced blood ACD levels 35 times higher than baseline levels. Similarly, blood ACD levels can be greatly augmented by antabuse treatment. In socially drinking individuals, treatment with antabuse increased blood ACD levels fivefold to tenfold compared to controls (Hald and Jacobsen 1948; Larsen 1948). Additionally, antabuse treatment rendered ACD detectable in the breath of human participants following alcohol exposure (Hald and Jacobsen 1948). Animal research soon elucidated the metabolic pathway of EtOH and replicated the observation that ACD could be detected in the breath of rabbits receiving antabuse and alcohol (Hald et al. 1949a, b).

Given the clinical implications of the early antabuse studies, several theories emerged associating alcoholism and ACD (Carpenter and MacLeod 1952; Davis and Walsh 1970; Griffiths et al. 1974; Myers and Veale 1969; Truitt and Walsh 1971). The most strident theories suggested that ACD was responsible for all of the effects associated with alcohol and that alcoholism would be more appropriately termed acetaldehydism (Raskin 1975; Truitt and Walsh 1971). Contrary studies suggested that ACD did not significantly mediate the effects of alcohol, and only trace amounts of ACD could be found in the cerebrospinal fluid or brain following alcohol consumption (Eriksson et al. 1980; Pikkarainen et al. 1979; Kiianmaa and

Virtanen 1978; Sippel 1974). These findings coupled with the observation that ACD could only cross the blood brain barrier at high concentrations (Eriksson 1977; Petersen and Tabakoff 1979; Sippel 1974; Tabakoff et al. 1976) suggested that ACD could not and did not contribute to the behavioral or pharmacological effects of alcohol. Subsequent evidence of the local formation of ACD within the brain research rejuvenated the theory that ACD could mediate the biological effects of EtOH consumption (Cohen et al. 1980).

1.2 Acetaldehyde Production in CNS

In the periphery, ACD is formed from EtOH through the action of ADH primarily in the liver. In the brain, ADH is inactive (Zimatkin et al. 1998), and formation of ACD from EtOH is achieved primarily through the action of another enzyme, catalase (Smith et al. 1997; Sippel 1974; Zimatkin 1991). Originally, it was postulated that peripheral ACD was unlikely to enter the CNS due to the prevalence of ALDH and the blood–brain barrier (Hunt 1996). Further research indicated that high levels of peripherally administered ACD results in detection of ACD in the brain within minutes (Ward et al. 1997) Therefore, peripheral ACD may overwhelm the peripheral ALDH, allowing some percentage of ACD to enter the brain (Quertemont et al. 2005). Additional local metabolic pathways (e.g., mitochondrial cytochrome P450) can also result in the formation of ACD from EtOH in the brain (Zakhari 2006). It is these non-ALDH metabolic pathways that produce the majority of ACD within the brain following EtOH consumption (Zimatkin et al. 1998).

2 Acetaldehyde Reactivity

ACD is a highly reactive compound that reacts with several endogenous catecholamines to form biologically active compounds (Cohen 1976; Cohen and Collins 1970; Davis and Walsh 1970; Walsh et al. 1970). With regards to research addressing alcohol use disorders, the compounds that have been of interest fall into two main classes: (1) the tetrahydroisoquinoline alkaloids (THIQs), which are formed through the direct and indirect interaction of ACD with monoamines (DA, epinephrine, and norepinephrine; Cohen 1976) and (2) the tetrahydrobetacarbolines (TBCs), which are formed through the condensation of ACD with the indoleamines (tryptophan and tryptamine; Buckholtz 1980). The most commonly investigated THIQs, tetrahydropapaveroline (THP), and salsolinol (SAL), have been detected in the brain following the administration of alcohol (Weiner 1980). The TBCs have also received attention as to their underlying role in alcohol-associated behaviors and the physiological effects of alcohol; contradictory data on the actions of TBCs has been reported.

2.1 Alcohol, Acetaldehyde, and Acetaldehyde Products

Research studies have attempted to establish that ACD is a necessary component for the manifestation of the neurobiological and behavioral aspects of alcohol use disorders. Such studies have made use of compounds that inhibit the formation of ACD or sequester ACD into a stable non-reactive adduct. Early studies utilized the compound 3-amino-1,2,4-triazole (triazole), which inhibits brain catalase activity, halting the primary pathway for the breakdown of alcohol in the brain. Triazole administration decreased alcohol consumption in rats and mice (Aragon and Amit 1992; Koechling and Amit 1994), reduced EtOH-related motor depression in rats (Aragon et al. 1985), and EtOH-stimulated motor activity in mice (Escarabajal et al. 2000). Triazole also decreased the intake of saccharin-quinine solution (Rotzinger et al. 1994) and food (Tampier et al. 1995) suggesting the compound could be causing non-specific effects unrelated to ACD formation as a result of alcohol exposure. Similar research has been completed using the compound D-penicillamine, which acts to sequester ACD into a non-reactive stable adduct but does not alter EtOH metabolism. Studies using D-penicillamine have established that sequestering ACD results in a reduction of alcohol intake and a decrease in alcohol conditioned place preference (CPP) in rats (Diana et al. 2008; Font et al. 2006b; Peana et al. 2008). D-penicillamine also reduces alcohol CPP and alcohol-induced motor depression in mice (Font et al. 2005, 2006a).

Research has focused on the contribution of the THIQs to physiological effects of alcohol and ACD. Early studies indicated that ACD acted to inhibit the metabolism of dopaldehyde alkaloid, which is formed from the condensation of dopamine and ACD, resulting in greater levels of THP (Davis and Walsh 1970). Central administration of low concentrations of THP into the lateral ventricles produced an increased preference and consumption of EtOH in rats and primates (McCoy et al. 2003; Melchior and Myers 1977; Myers and Melchior 1977). Higher doses of THP resulted in decreased alcohol preference and consumption (Duncan and Deitrich 1980). Microinjections of lower doses of THP into regions in the mesolimbic reward pathway, including the VTA or the Acb, acted to increase alcohol preference in rats (Myers and Privette 1989; Duncan and Fernando 1991). Research has attempted to elucidate the neurochemical systems mediating the effects of THP on EtOH consumption (Myers and Privette 1989; Privette and Myers 1989). Buspirone (a 5-HT 1a receptor agonist) decreased the THP-stimulated augmentation of EtOH consumption (Privette et al. 1988). Further research with THP has not been conducted.

Systemic administration of SAL increased EtOH consumption and preference (Myers and Melchior 1977). The ability of SAL to augment EtOH consumption is centrally mediated since microinjections of SAL into the lateral ventricle increased EtOH consumption and preference (Purvis et al. 1980). Similar to ACD, research has indicated that SAL possesses reinforcing/rewarding properties. Peripherally administered SAL will condition a place preference (Matsuzawa et al. 2000).

Compared to other ACD products, the neurochemical basis of SAL effects has been studied more intensively. SAL inhibits catecholamine reuptake (Alpers et al. 1975; Heikkila et al. 1971; Tuomisto and Tuomisto 1973) and/or metabolizing enzymes such as catecholmethyltransferase and monoamine oxidase (Alpers et al. 1975; Collins et al. 1973) resulting in increases in catecholamine levels. SAL has been shown to significantly decrease striatal levels of 5-HT metabolic enzymes, subsequently increasing 5-HT to a level 20-fold higher than DA (Nakahara et al. 1994). Additionally, SAL has been shown to possess a high affinity for the μ opioid receptor (MOR) (Airaksinen et al. 1984). SAL-induced locomotor activity, CPP, and stimulated DA release in the AcbSh can be reduced by microinjections of MOR antagonists into the posterior VTA (Hipolito et al. 2010, 2011).

There has been less attention on the role of TBCs in alcohol use disorders compared to ACD or the THIQs. Initial studies indicated that acute peripheral administration of TBC derivatives acted to decrease the preference for alcohol over water in rats (Geller and Purdy 1975; Geller et al. 1973; Messiha and Geller 1976). Chronic intra-cerebral ventricular (ICV) microinjections of the TBC tryptoline significantly increased alcohol preference and consumption (Adell and Myers 1994; Airaksinen et al. 1983; Huttunen and Myers 1987; Myers and Melchior 1977; Tuomisto et al. 1982). Co-infusion of tryptoline and THP augmented alcohol preference and consumption in a synergistic nature (Myers and Oblinger 1977). Infusion of TBC into the hippocampus of low alcohol drinking (LAD) rats increased alcohol preference and consumption (Adell and Myers 1995; Huttunen and Myers 1987) through increases in both 5-HT and norepinephrine levels (Adell and Myers 1995). TBCs exhibit an affinity for the Delta opioid receptor (DOR) (Airaksinen et al. 1984), but the pharmacological properties of TBCs have not been fully examined.

3 Acetaldehyde is Pharmacologically Active in the CNS

Intra-cerebral ventricular (ICV) microinjections of ACD increased preference for and consumption of alcohol in rodents (Brown et al. 1979, 1980). It was also discovered that ACD possessed reinforcing properties as rats would readily self-administer ACD through both ICV (Amit et al. 1977; Brown et al. 1979, 1980) and intra-venous (IV) (Myers et al. 1984) routes. Animals receiving ICV infusions of ACD exhibited a CPP associated with the drug (Smith et al. 1984) while peripheral injections of ACD induced a conditioned taste aversion similar to alcohol (Aragon et al. 1986).

Central and peripheral administration of ACD produces a CPP in a variety of rat lines (Quintanilla and Tampier 2003; Spina et al. 2010). Quertemont and De Witte (2001) reported that rats showed a dose-dependent stimulus preference when ACD was administered peripherally. Much like EtOH, ICV-administered ACD at lower doses, produced an elevation in locomotor activity in rats (Correa et al. 2003),

while peripheral and central administration of high doses of ACD produced motor depression in both rats and mice (Durlach et al. 1988; Holtzman and Schneider 1974; Myers et al. 1987; Quertemont et al. 2004; Tambour et al. 2006). Similar biphasic effects on locomotor activity have been observed following vapor exposure to ACD (Ortiz et al. 1974).

While there is still dispute over the extent to which ACD contributes to the neurobiological and behavioral actions of alcohol, emerging evidence indicates that ACD is biologically active and may mediate, in part, alterations in behavior produced by EtOH exposure/consumption. Research has shown that both central and peripheral administration of ACD cause an increase in alcohol consumption in rats (Brown et al. 1979, 1980). Rats will exhibit ACD-induced CPP and stimulus preference suggesting that ACD is rewarding (Quertemont and De Witte 2001; Quintanilla and Tampier 2003; Smith et al. 1984; Spina et al. 2010); blocking or sequestering the formation of ACD resulting from alcohol exposure produces alterations in the neurobiological and behavioral effects of alcohol (Aragon and Amit 1992; Diana et al. 2008; Font et al. 2005, 2006a, b; Kaharanian et al. 2011; Koechling and Amit 1994; Peana et al. 2008). While it is currently difficult to assert that ACD is absolutely necessary for the neurobiological and behavioral actions of alcohol, data show that ACD is likely to be involved to some extent.

3.1 Acetaldehyde in the VTA: In Vivo Electrophysiology

ACD has excitatory actions on neurons of the VTA as clearly demonstrated by the effects on dopamine release and on the firing frequency of individual VTA neurons. In experiments using in vivo recording methods, ACD was injected intravenously at doses from 5 to 40 mg/kg, and a dose-dependent increase in firing of dopaminergic VTA neurons was reported (Foddai et al. 2004). Thus, ACD parallels the effects observed with EtOH, but at 50 times lower concentrations. The effects of EtOH on VTA neuronal activity was blocked by systemic pretreatment with the ADH inhibitor 4-methylpyrazole, but this drug had no effect on ACD-induced excitation (Foddai et al. 2004), suggesting that the excitatory effects of EtOH on the VTA are mediated by ACD. The data also implicated that peripheral ACD formation (mediated by ADH) rather than central ACD formation (which would be mediated by catalase) was the basis of the finding. Sequestration of ACD by in vivo administration of D-penicillamine is sufficient to block the excitatory effects of intragastrically administered EtOH or intragastrically administered ACD (Enrico et al. 2009).

The clear effects of manipulation of ACD by a variety of methods (e.g., enzyme antagonism or ACD sequestration) indicate that this is a robust in vivo phenomenon, and the effect of EtOH on dopaminergic VTA neurons in vivo is dependent on ACD.

3.2 Acetaldehyde in the VTA: In Vitro Studies

Significant research has been performed using brain slice preparations showing that acutely applied ACD can increase the firing frequency of dopaminergic VTA neurons. The key results of that study indicate that ACD-induced activation of dopaminergic VTA neurons mimics EtOH-induced excitation (Diana et al. 2008), and is produced at much lower concentrations (10–100 nM) compared to EtOH (typical excitatory concentrations of 20–120 mM; Brodie and Appel 1998; Brodie et al. 1990). Furthermore, EtOH applied in the presence of a catalase inhibitor, 3-aminotriazole (1 mM), failed to produce its characteristic excitation of the VTA neurons in this study. In exploring the mechanism of ACD excitation of VTA neurons, Melis et al. (2007) examined the effect of ACD on two ion currents, A-current and h-current. An A-current represents a rapidly-inactivating potassium current that can contribute to spike after hyperpolarization and is involved in the regulation of firing frequency of dopaminergic VTA neurons (Koyama and Appel 2006a). On the other hand, h-current is a characteristic current of dopaminergic VTA neurons that is activated at membrane potentials about 20 mV negative to the resting membrane potential; it may contribute to the spontaneous firing rate of dopaminergic VTA neurons from mice (Okamoto et al. 2006; McDaid et al. 2008) but not those from rats (McDaid et al. 2008; Appel et al. 2003), but its major role is likely to be in the regulation of excitability and synaptic signal integration in dopaminergic VTA neurons (Inyushin et al. 2010). The authors noted a right-ward voltage shift produced by ACD on I_A (Melis et al. 2007). Also noted was a significant increase in h-current produced by acutely applied ACD; this is consistent with an effect of EtOH, which has been shown to acutely increase I_h of VTA neurons in brain slices (Brodie and Appel 1998; Okamoto et al. 2006). In addition, the authors blocked ACD-induced excitation with two specific ion channel blockers: 4-aminopyridine (10 mM) which blocks A-current and ZD7288, which blocks h-current. Both agents apparently blocked the ACD-induced excitation of the VTA neurons (Melis et al. 2007). The firing rate was increased by 4-aminopyridine alone, and no further increase in firing was observed with the addition of ACD. In contrast, ZD7288 (30 μM) alone reduced the firing rate of dopaminergic VTA neurons (as has been seen by some (Okamoto et al. 2006) but not others (Appel et al. 2003), and no ACD-induced excitation was observed in the presence of ZD7288.

The most parsimonious model suggests that EtOH is metabolized to ACD by catalase locally in the VTA, and the authors of these studies suggest that, in general, EtOH actions on the VTA are mediated by ACD.

3.3 Differences in the Action of Acetaldehyde: Methodological Considerations

It is an inherently unsatisfying argument to invoke methodological differences to explain contrary experimental results, but in the case of brain slice

electrophysiology, often this is the underlying source of controversy. Variables such as ionic concentrations in external or internal media, purity of reagents, species, strain, or supplier of subjects, and even plane of section, can alter the results and can lead to different conclusions among investigators. Controversies in the literature among investigators may not be amenable to resolution simply due to the multiplicity of unknown variables. Each laboratory achieves consistency of results by controlling many of these variables, but each may control these variables differently. With ACD, the differences among laboratories may become more extreme, as the reactivity and labile chemical nature of ACD may yield a null result, even when other variables are controlled. Parasaggital section of the midbrain may yield slices with relatively more intact glutamatergic fibers than coronal sections; ACD has been shown to interact with glutamatergic systems (Padilla-de la Torre et al. 2008). There is ample evidence that ZD7288 reduces GLU release as well as blocking h-current; if a portion of ACD effects were produced by actions on presynaptic glutamatergic endings in the VTA, differences in the viability of the GLU terminals could explain differences in the effects of ACD and other agents.

3.4 Differences Between Acetaldehyde and Alcohol: Electrophysiological Studies

While the results of these studies, especially those of the enzyme antagonist experiments, indicate that EtOH actions on the firing of mesolimbic dopaminergic neurons are mediated by ACD formed in the VTA, there are some clear differences in the mechanisms of action of the two agents. ACD-induced excitation of dopaminergic VTA neurons appears to be mediated by effects on h-current and A-current (Melis et al. 2007). EtOH excitation in dopaminergic neurons of the rat has been shown to be blocked by quinidine, but not by blockers of h-current (Appel et al. 2003). Furthermore, EtOH excites individual VTA neurons dissociated from brain slices (Brodie et al. 1999; Ye et al. 2001), a preparation that would be expected to reduce the ability of synthesized ACD to act on these independent VTA neurons, and the concentration response for EtOH excitation of dissociated dopaminergic VTA neurons (Brodie et al. 1999) is similar to that observed in brain slices (Brodie et al. 1990).

Clearly, the results of Melis et al. indicate that ACD affects both A-current and h-current, but these effects are not consistent with an EtOH-like action. Acute (Brodie and Appel 1998) and chronic (Okamoto et al. 2006) EtOH has clear effects on h-current, and it suggests that some of the effects of EtOH on firing frequency should be mediated by h-current. Despite the finding in one study that suggested that EtOH excitation of dopaminergic VTA neurons of mice could be blocked with ZD7288 (Okamoto et al., 2006), additional studies of this phenomenon indicate that apparent reduction of EtOH excitation was more likely due to effects of ZD7288 that are not related to its action on h-channels (McDaid et al. 2008). The role of h-current in modulating the firing frequency of dopaminergic neurons of the

VTA may differ in different preparations and in different rodents, as ZD7288 alone produces a decrease in firing rate in some studies (Melis et al. 2007; Okamoto et al. 2006), but not in others (Appel et al. 2003) except at high concentrations (Seutin et al. 2001). Under conditions and in species in which ZD7288 does not affect the firing rate, it also does not affect EtOH action. The action of ZD7288 and other blockers of ion channels may depend on specific experimental conditions.

One agent that has been shown to decrease EtOH excitation in rat dopaminergic VTA neurons is quinidine (Appel et al. 2003), which was not tested against ACD-induced excitation. EtOH also decreases M-current (Koyama et al. 2007), and the effect of ACD on M-current is unknown. M-current is a voltage dependent, sustained potassium current that affects the firing frequency of dopaminergic VTA neurons (Koyama and Appel 2006b). A study of the effects of EtOH on the ion channel responsible for M-current is one example of the difficulty in cataloging the effects of agents on ionic currents and then postulating a functional role for those currents on cell activity. EtOH reduces M-current of dopaminergic VTA neurons in a concentration-dependent manner (Koyama et al. 2007), but the selective M-current blocker XE-991 did not significantly reduce EtOH-induced excitation (Koyama et al. 2007). The effects of EtOH on M-current may be physiologically important in some processes (for example, adaptation to chronic EtOH exposure) yet modulation of M-current does not alter the acute excitatory effect of EtOH.

The data indicate the EtOH and ACD are not producing the same electrophysiological effects on VTA DA neurons, but the results of studies indicating the lack of an EtOH effect on dopaminergic neurons in the presence of antagonists of ADH (Foddai et al. 2004) or catalase (Melis et al. 2007; Diana et al. 2008) are compelling, and suggest that the metabolism of EtOH to ACD mediate EtOH-induced excitation in dopaminergic VTA neurons. It seems most likely that ACD is a crucial component of the overall effects of EtOH on dopaminergic neurons of the VTA; the essential action of ACD could be parallel to EtOH, or it could enhance EtOH-induced changes. Blockade of the formation of ACD can reduce the response of dopaminergic VTA neurons to EtOH, and could serve as a platform for the development of agents that reduce the rewarding and reinforcing actions of EtOH.

3.5 Behavioral Pharmacology of Acetaldehyde Within the Mesolimbic Dopamine System

Despite the inconsistent electrophysiological studies, the results of behavioral pharmacological research examining the affects of ACD within the mesolimbic dopamine (VTA and Acb) have been relatively consistent. EtOH, ACD, and SAL are directly self-administered into the posterior, but not anterior, VTA (Rodd et al. 2008, 2005; Rodd-Henricks et al. 2002). Self-administration of all three compounds into the posterior VTA can be extinguished by co-administrations of

quinpirole, a D_2 receptor agonist (Rodd et al. 2005, 2008). Thus, the reinforcing actions of all three compounds in the posterior VTA is dependent upon VTA DA neuronal activity, since quinpirole would act to stimulate D_2 autoreceptors (reduction in DA activity). SAL microinjected into the VTA will produce a CPP (Hipolito et al. 2010, 2011). Reverse microdialysis of SAL has been shown to modulate DA levels within the Acb (Hipolito et al. 2009). EtOH and SAL self-administration, but not ACD, directly into the posterior VTA is extinguished by co-administration of 5-HT receptor antagonists (Rodd et al. 2005, 2008). EtOH has direct actions on 5-HT_3 receptors (Lovinger and White 1991) and SAL can increase the release of 5-HT (Maruyama et al. 1992, 1993), but ACD does not express an affinity for 5-HT_3 receptors (Li 2000).

Examining the concentration of SAL, ACD, and EtOH required to support self-administration directly into the posterior VTA provides an indication of the efficacy between the compounds. EtOH is typically self-administered between the ranges of 20–80 mM directly into the posterior VTA (Rodd et al. 2003, 2005). ACD is reinforcing between 6 and 90 μM, while SAL is self-administered between 0.03 and 0.3 μM directly into the posterior VTA (Rodd et al. 2005, 2008). Thus, the concentration required for SAL to support self-administration into the posterior VTA is 200-fold less than that required for ACD and 300×10^3 lower than EtOH. All of these concentrations are within the range observed in the brain following oral consumption of EtOH (Haber et al. 1997, Zimatkin et al. 1998), and are thus pharmacologically relevant.

In addition, the dose response curves of the three compounds would support the stance that EtOH is possibly a pro-drug. However, EtOH self-administration directly into the posterior VTA is not altered by co-administration of a catalase antagonist (3-amino-1,2,4-triazole; triazole; Rodd et al. 2005). Yet, ACD could have been produced within the posterior VTA following self-infusion of EtOH through a catalase-independent pathway. A more recent study has examined the possible role for ACD in the etiology of alcohol use/abuse through the use of lentiviral vectors that decrease catalase activity or increase ADH activity in the VTA (Kaharanian et al. 2011). Administration of either lentiviral vector into the VTA acted to decrease voluntary consumption of EtOH as well as EtOH-stimulated DA release in the AcbSh suggesting that the breakdown of EtOH into ACD is a component of EtOH reward (Kaharanian et al. 2011). A later study replicated such findings in that an anti-catalase viral vector was once again successful in decreasing EtOH intake in EtOH-naive rats (Quintanilla et al. 2011). However, the anti-catalase viral vector was only successful at decreasing EtOH consumption in animals that had previous access to EtOH over the course of 60 days, following a period of imposed abstinence (i.e., during relapse; Quintanilla et al. 2011).

The ability of a compound to stimulate VTA DA neurons can also be measured by determining the effects of compounds directly applied to the VTA on DA release in downstream projection areas. The effects of DA release in the Acb following microinjections of drugs into the VTA have been examined for EtOH, ACD, and ACD products. Myers and Robinson (1999) were first to report that THP microinjected into the anterior VTA had a direct effect on DA release in the Acb.

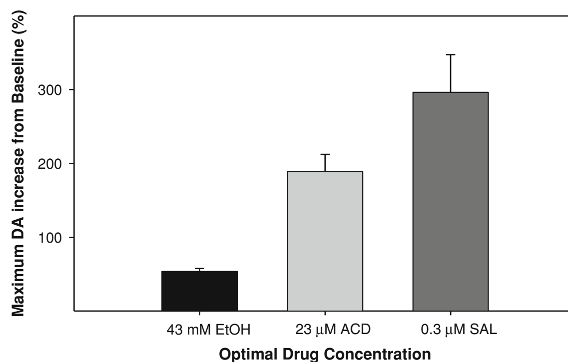

Fig. 2 Maximal dialysate levels of dopamine (DA) in the nucleus accumbens shell (AcbSh) as a result of microinjections of 43 mM ethanol (EtOH), 23 μM acetaldehyde (ACD), or 0.3 μM salsolinol (SAL) into the posterior ventral tegmental area (pVTA)

Further, THP was detected in vivo in the striatum (Haber et al. 1997) and Acb (Baum et al. 1999) showing that THP could be formed in the brain within structures involved in drug reward.

Recent studies have utilized the same equipment employed for intracranial self-administration studies to determine the effects of microinjection of a compound into the posterior VTA on DA levels in the AcbSh. Microinjections of 200 mg% EtOH (optimal concentration) into the posterior VTA increased DA levels in the AcbSh (Ding et al. 2009, 2011). Similar to the intracranial self-administration data, lower concentrations of ACD (23 μM) and SAL (0.3 μM) microinjected into the posterior VTA were able to evoke dopamine release in the AcbSh (GA Deehan et al. unpublished). The data sets also reveal that SAL was able to increase DA levels in the AcbSh at a greater amount than EtOH (Fig. 2). Therefore, there were parallel findings between the concentration required to produce reinforcement and that required to stimulate VTA DA neurons and evoke DA release in the AcbSh.

The research examining the effects of SAL and ACD in other brain regions has lagged behind that conducted in the VTA. Both EtOH and SAL are self-administered into the AcbSh, but not AcbC (Engleman et al. 2009; Rodd et al. 2003). Similar to the VTA data set, SAL was self-administered into the AcbSh at significantly (400×10^3) lower concentrations than EtOH. Both EtOH and SAL self-administration into the AcbSh could be extinguished by co-administration of a $D_{2/3}$ antagonist (sulpiride). Therefore, the reinforcing properties of both SAL and EtOH within the AcbSh are dependent upon activation of post-synaptic DA receptors. The reinforcing properties or neurochemical effects of EtOH, ACD, and SAL in other brain regions (e.g., mPFC or central amygdala) have not been extensively studied.

4 General Summary

The current literature indicates that the reinforcing properties, and the behavioral consequences, of EtOH are mediated, in part, by ACD and ACD products (i.e., SAL). In general, the data reflect the complexity of EtOH within the CNS.

There is evidence that EtOH can directly act at receptors and to stimulate VTA DA neurons (Brodie et al. 1999; Ye et al. 2001; Lovinger and White 1991). Convergent evidence that both ACD and SAL have distinct actions within the mesolimbic dopamine system has recently been reported. Additional studies have indicated that EtOH consumption and reinforcement may be mediated by the conversion of EtOH into ACD. The following statements are supported by current research; (1) EtOH can have direct actions to produce neurochemical, electrophysiological, and behavioral consequences, (2) some of EtOH's actions may be mediated, in part, by the conversion of EtOH into ACD, (3) ACD in the CNS has reinforcing properties which are mediated by the mesolimbic dopamine system, (4) products derived from ACD can also produce reinforcement within the mesolimbic dopamine system, (5) the actions of EtOH, ACD, and SAL within the mesolimbic dopamine system can occur at physiologically relevant levels, and (6) whole areas of research about the EtOH-ACD-SAL system has not been elucidated. Ultimately, the sequelae of alcoholism may be based upon a complex series of interwoven peripheral and central effects of EtOH and its metabolites. It is perhaps our lack of understanding of this complex system that has prevented the development of successful pharmacotherapeutics for the treatment of alcoholism.

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Part II
Genetics

Modeling the Diagnostic Criteria for Alcohol Dependence with Genetic Animal Models

John C. Crabbe, Kenneth S. Kendler and Robert J. Hitzemann

Abstract A diagnosis of alcohol dependence (AD) using the DSM-IV-R is categorical, based on an individual's manifestation of three or more symptoms from a list of seven. AD risk can be traced to both genetic and environmental sources. Most genetic studies of AD risk implicitly assume that an AD diagnosis represents a single underlying genetic factor. We recently found that the criteria for an AD diagnosis represent three somewhat distinct genetic paths to individual risk. Specifically, heavy use and tolerance versus withdrawal and continued use despite problems reflected separate genetic factors. However, some data suggest that genetic risk for AD is adequately described with a single underlying genetic risk factor. Rodent animal models for alcohol-related phenotypes typically target discrete aspects of the complex human AD diagnosis. Here, we review the literature derived from genetic animal models in an attempt to determine whether they support a single-factor or multiple-factor genetic structure. We conclude that there is modest support in the animal literature that alcohol tolerance and withdrawal reflect distinct genetic risk factors, in agreement with our human data. We suggest areas where more research could clarify this attempt to align the rodent and human data.

J. C. Crabbe (✉) · R. J. Hitzemann
Portland Alcohol Research Center, Department of Behavioral Neuroscience,
Oregon Health & Science University, Portland, OR 97239, USA
e-mail: crabbe@ohsu.edu

K. S. Kendler
Virginia Institute for Psychiatric and Behavioral Genetics,
Virginia Commonwealth University, Richmond, VA, USA

J. C. Crabbe · R. J. Hitzemann
VA Medical Center, Portland, OR 97239, USA

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Abbreviations

AA/ANA	Alko Alcohol/Nonalcohol rat selected lines
AD	Alcohol dependence
ADH	Alcohol dehydrogenase
AFT	Acute functional tolerance
ALDH	Aldehyde dehydrogenase
BEC	Blood ethanol concentration
BLA	Basolateral amygdala
BXD RI	Recombinant inbred strains derived from crossing C57BL/6J and DBA/2J inbreds
C57BL/6J	A common inbred strain of mice
CA3	Region of hippocampus
CeA	Central nucleus of the amygdala
DBA/2J	A common inbred strain of mice
DSM-IV-R	Diagnostic and Statistical Manual of the American Psychiatric Association
FHP/FHN	Family history positive/negative
GABA	Gamma aminobutyric acid
GLAST	Gene encoding the glutamate-aspartate transporter
GLT-1	Gene encoding a glutamate transporter
GluR1,2,4	Genes encoding glutamate receptor subunits
Gnb1	Gene encoding the guanine nucleotide binding protein beta 1 subunit
GO	Gene ontology
HAFT/LAFT	High/Low Acute Functional Tolerance mouse selected lines
HAPLAP	High/Low Alcohol Preferring mouse selected lines
HDID	High Drinking in the Dark mouse selected line
HIC	Handling-induced convulsion
HT	Hypothermia
HRT/LRT	High/Low Rapid Tolerance mouse selected lines
NAc	Nucleus accumbens
P/NP	Preferring/Non-preferring rat selected lines
QTL	Quantitative trait locus/loci
Scd5	Gene encoding a stearoyl-CoA desaturase isoform
Scn4b	Gene encoding the sodium channel 4b subunit
SNP	Single nucleotide polymorphism
WDR	Withdrawal
WGCNA	Weighted gene covariance network analysis
WSP/WSR	Withdrawal Seizure-Prone/-Resistant mouse selected lines

Contents

1 Introduction 189
2 Goal of the Review and Method of Analysis 190
3 Three Distinct Clusters of Genetic Risk Influence Alcohol Dependence Diagnosis on DSM-IV-R 191
4 Consilience of Animal Phenotypes and Human Diagnostic Criteria 192
4.1 Tolerance 192
4.2 Withdrawal Severity 195
5 Rodent Methods for Assessing Genetic Correlation 196
6 Evidence for Genetic Correlation Across AD Criteria in the Rodent Literature 197
6.1 Selected Lines 198
6.2 Standard Inbred Strains 200
6.3 Recombinant Inbred Strains 202
6.4 Summary of Rodent Data 202
7 Consilience in Studies of Gene Expression 203
7.1 Human Expression Data from Post Mortem Brain Tissue 205
7.2 Rodent Expression Studies 206
7.3 An Alternative Strategy for Relating Gene Expression to Function 208
8 Conclusions and Future Directions 209
9 Appendix 211
References 214

1 Introduction

In the US, alcohol dependence (AD) is diagnosed using criteria set out in the Diagnostic and Statistical Manual-IV-R (DSM-IV-R) of the American Psychiatric Association. Seven criteria are evaluated, and if an individual displays any three or more of them during the same 12-month period, he or she meets the diagnostic criteria. The seven criteria are: tolerance (more must be drunk to achieve the desired effect, or drinking the same amount produces a much diminished effect), withdrawal (symptoms appear when drinking is discontinued), loss of control (drinking cannot be modulated once started), desire to quit (drinking is initiated despite desire not to drink), preoccupation (excessive thoughts and activities pertaining to gaining access to or consuming alcohol), activities curtailed (other activities are reduced as time is dominated by drinking and recovering), and persistence (drinking continues despite medical and/or social consequences). The other diagnostic scheme in wide use, the International Classification of Diseases (ICD)-10, is similar in its categorical approach to diagnosis and congruence between diagnoses using the two systems is high.

There is a substantial genetic contribution to risk for AD. Twin, adoption and other studies suggest that 50–60% of individual differences in risk are heritable (Enoch and Goldman 2001; Goldman and Ducci 2007). However, there is no consensus on whether AD diagnosis represents a single versus a multifactorial genetic phenotype. Some studies suggest that there are different subtypes of alcoholism with distinct genetic architectures, with many possible subtyping

schemes proposed [e.g. (Cloninger 1987)]. Recently, the contributions of the individual DSM-IV-R criteria have been suggested to represent a single continuum of underlying risk (Grant 2000), perhaps primarily reflecting heavy consumption (Grant et al. 2009). Most gene finding studies have used the categorical diagnosis of AD, assuming that the linkage or association of variation in candidate genes or genetic markers is generally informative for diagnostic risk [e.g. (Edenberg and Foroud 2006; Prescott et al. 2006; Treutlein and Rietschel 2011)]. Many individuals have positive scores on more than one of the seven diagnostic criteria for AD. In one epidemiological study, one-fourth of AD individuals had positive scores on 5, 6, or all 7 criteria (Grant 2000).

Genetic contributions to individual differences in the degree of responses related to alcohol have been studied in animal models for many years. It was established in the 1940s that genes led some rats to prefer to drink a 10% alcohol solution more than others (Mardones and Segovia-Riquelme 1983) and in 1959 that different inbred strains of mice had genetic proclivities ranging from strong preference (such as the C57BL/6J strain) to near abstinence (such as the DBA/2J strain) (McClearn and Rodgers 1959). Genetic animal models have been a major influence on alcoholism research since then, and their contributions have been reviewed elsewhere (Crabbe 2008; Edenberg and Foroud 2006). Some genotypes of rats and mice have been developed through selective breeding for high two-bottle ethanol preference drinking. Preferring (P), Alko Alcohol (AA) and several other rat lines (and High Alcohol Preferring—HAP—mouse lines) drink a substantial amount of alcohol during each 24 h day, especially as compared with NP (ANA, LAP and others) selected for low drinking (Crabbe et al. 2010b). Some argue that the genetic high preferrers represent an animal model of alcoholism (Bell et al. 2006; Sommer et al. 2006). However, they do not usually drink enough to become intoxicated at any point during the day. Nor do they show pronounced tolerance or withdrawal after voluntary drinking. Thus, we have argued that these selected lines of animals do not constitute a model of the complex AD diagnosis, but rather capture genetic contributions to select aspects of alcohol drinking (Crabbe 2008; Crabbe et al. 2009). If genetic animal models cannot capture the entire range of such a complex genetic trait as AD, a more reasonable goal when designing such a model is to target specific alcohol-related responses (McClearn 1979); indeed, this has been the practical goal followed during the development of each such model. Rodent lines have also been selected for high versus low genetic susceptibility to alcohol intoxication/sedation, locomotor stimulation, tolerance, and withdrawal severity (Browman et al. 2000; Crabbe 2008).

2 Goal of the Review and Method of Analysis

The goal of this paper is to review how data from rodent models inform the debate regarding whether AD represents one or multiple underlying genetic factors. Specifically, can the various rodent genotypes that have been used to study

alcohol's effects provide evidence for or against the single-factor AD risk hypothesis? In a recent analysis of twin data (see Sect. 3), we found that the diagnostic criteria for DSM-IV-R AD diagnosis represent three somewhat distinct genetic paths to individual risk (Kendler et al. in review). If an animal known to be genetically susceptible to one effect of alcohol also proves to be genetically susceptible to another, this suggests that the two traits may share common genetic determinants—i.e. are genetically correlated. Thus, in genetic terms, we will review the animal evidence for genetic correlation, where genes exert pleiotropic effects on multiple phenotypes. Specifically, if a rodent that is genetically high-scoring on one criterion of an AD diagnosis also scores high on many or most others, we would take this as evidence favoring a single genetic factor model for the construct. An informative rodent study would need to be able to discriminate a genetic from an environmental source for that correlation. Alternatively, if the pattern of genetic influences across criteria in rodents paralleled the human data, we would take this as supporting the multiple factor model. In the twin paper, we suggested that the animal data provided partial support for the human genetic architecture (Kendler et al. in review). Here, we discuss the animal data in more depth.

In this review, we first discuss the behaviors themselves and the similarities and differences between the human criteria and their counterpart animal assays. The degree to which rodent behaviors are consilient with the human symptoms they attempt to model presents a difficult problem (Cicero et al. 1979; Crabbe 2010). We then describe the most powerful animal genetic methods available for comparing the animal and human genetic data. We next explore the genetically informative animal data in more detail. Finally, we review the most relevant genetic data from animal models based not on the allelic differences among individuals, but on the differential expression of genes. We conclude that the three-factor model for the human data is broadly consistent with the majority of the animal data. We also identify areas where there could be substantial improvements made in providing discrete models for some of the human diagnostic criteria, and give examples of how those new models could be used to test the hypothesis further.

3 Three Distinct Clusters of Genetic Risk Influence Alcohol Dependence Diagnosis on DSM-IV-R

In a recent multivariate twin analysis of interview data from 7,548 adult twins from the Virginia adult twin study of psychiatric and substance use disorders, we used structural equation modeling to clarify the structure of the genetic and environmental risk factors for each of the seven individual criteria for AD diagnosis. Also included in the model were two screening items, positive response to which was necessary for entry into the alcohol section of the interview. The best fit model included three genetic common factors, two unique environmental common factors and environmental factors unique to each criterion (Kendler et al. in review).

We termed the genetic risk factors: (a) heavy use and tolerance (loading on the first screening item reflecting excess quantity or frequency of alcohol consumption and the tolerance criterion); (b) loss of control with alcohol associated social dysfunction (which loaded heavily on loss of control, desire to quit, preoccupation, and activities given up); and (c) withdrawal and continued use despite problems. We do not consider the environmental risk factors here.

4 Consilience of Animal Phenotypes and Human Diagnostic Criteria

Of the seven criteria for an AD diagnosis in DSM-IV-R, not all can reasonably be modeled in rats or mice. It has been realized for many years that the principal strength of rodent genetic animal models is to produce partial models for complex human traits (McClearn 1979). Research into the genetics of alcoholism is rich in both human and rodent data, but most researchers work with humans only, or with one or the other rodent species. Perhaps as a consequence, the behaviors studied in rodent laboratories often do not completely resemble their human counterparts. A recent effort to address the problems of better consilience between human behaviors and laboratory rodent behavioral targets focused on alcohol-related traits (Crabbe 2010) and considered several features of human alcoholism in detail, comparing several aspects of risk and comorbidity. The reviews resulting from this effort also focused on the genetic correlations among different traits (Ehlers et al. 2010; Stephens et al. 2010; Sher et al. 2010; Crabbe et al. 2010a; Heilig et al. 2010; Dick et al. 2010; Leeman et al. 2010).

We consider first each of the seven criteria and discuss whether the rodent behavioral assays plausibly parallel the human diagnostic criterion. We limit the animal discussion to the data from rodents, as they comprise the majority of the genetic animal model work. Desire to quit, preoccupation, and loss of control have not been modeled in rodents, and we believe it unlikely that a rodent parallel for these self-report measures exists. Similarly, "activities given up" has not been modeled directly. Continued use despite problems could perhaps be approached through certain animal behavioral assays, but no relevant genetic data currently exist to our knowledge. Thus, we set aside these five of the seven criteria. Withdrawal and tolerance have clear laboratory animal parallels. Given the clear distinction from the human data between withdrawal and tolerance, the preponderance of relevant data from animals is adduced to trying to ascertain whether the animal data support commonality of genetic influence or lack thereof on tolerance and withdrawal.

4.1 Tolerance

Drug tolerance is defined as the reduction of response intensity or duration after chronic administration; alternatively, it is defined as the requirement to raise the dose of a drug in order to maintain an initial level of response (Kalant et al. 1971).

There are two mechanistically distinguishable types of tolerance, pharmacodynamic (functional) and pharmacokinetic (metabolic). In functional tolerance, the amount of drug and/or active metabolite that remains in contact with the effector tissue has not changed, but the target tissue no longer responds in the same way. For example, we assume that many of alcohol's behavioral effects are due to interactions with brain, and in a tolerant individual, certain receptors may no longer signal their intracellular partners as effectively. Metabolic tolerance occurs when the processes of drug absorption, distribution among body compartments (e.g., brain, blood, soft tissues), metabolism to other chemicals, and/or excretion lead to a significant reduction in the amount of alcohol in the body—specifically, at the effector tissue. Given alcohol's pharmacokinetics, this can occur if alcohol's metabolic enzymes, primarily alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH), have been induced to work more actively.

Both types of tolerance occur with alcohol. For humans, alcohol metabolism is involved with risk of AD. Polymorphisms in ADH and ALDH enzymes have provided the clearest evidence of an individual gene's important role in risk for an AD diagnosis. Many individuals of East Asian descent possess polymorphisms in ALDH that lead to slow elimination of acetaldehyde, a toxic metabolite. The circulating acetaldehyde in turn causes symptoms including facial flushing, nausea, dizziness, and headache, and these individuals have a clear lowered risk of developing AD (Chen et al. 1999; Enoch and Goldman 2001). However, the development of metabolic tolerance (i.e., more rapid elimination of alcohol with chronic use) is not thought to be an important factor in progression to an AD diagnosis.

Functional tolerance, on the other hand, plays a role in humans and is one of the diagnostic criteria for AD. The DSM-IV-R criterion for tolerance is typically assessed with two questions similar to the following: (1) Did you ever find that you needed to drink a lot more in order to get the same effect as you did when you first started drinking? And (2) Did you ever find that when you drank the same amount it had much less effect than before? Follow-up questions would then assess the actual amount of increased alcohol required to get the "same effect."

One way that tolerance has featured in analyses of genetic risk factors derives from the findings of Marc Schuckit's group and others beginning in the early 1980s. Family history positive (FHP) individuals were known to be at greater risk for AD than Family history negative (FHN) subjects. Schuckit's group brought young FHP and FHN men into the laboratory and gave them an alcohol challenge. He found that FHP subjects reported less sedation, body sway and felt less "high" than FHN subjects. They also showed blunted hormone responses. Following these subjects over the years revealed that so-called "low level of response" to alcohol predicted eventual AD diagnosis even better than FHP versus FHN status (Schuckit and Smith 1996; Schuckit 2000). However, the pattern of lower level of response in FHN subjects was not always seen by other investigators. A review of the literature suggested that so-called "low level of response" probably depends upon when the measurement is taken. If taken early after alcohol administration, FHP subjects actually show *enhanced* responses to alcohol relative to FHN for some measures, but if assessed an hour or more after ingestion, FHP responses

tend to be lesser than FHN (Newlin and Thomson 1990). This interpretation suggests that low level of response actually represents the more rapid development of acute functional tolerance (AFT) during the test session by FHN subjects.

In rats and mice, substantial metabolic tolerance does not normally occur if animals are drinking alcohol chronically unless there is no water available. Several procedural manipulations can be performed that increase rodents' oral intake of ethanol solutions, but these are typically labor-intensive and many require weeks if not months of access before animals will drink enough alcohol that they will display metabolic tolerance. Thus, differences in metabolic tolerance to ethanol are not normally a consideration for interpreting most animal studies.

To produce functional tolerance, rodents need to be given repeated injections or gastric intubations of alcohol. To produce greater levels of functional tolerance, animals may be fed a liquid diet, where alcohol solutions with added vitamins and minerals are substituted for food and water. Alternatively, they may be exposed to alcohol chronically by being placed in a chamber where alcohol vapor is provided and thus be chronically dosed by inhalation.

To measure functional tolerance in rodents, the usual practice is to study a sedative or intoxicating response. Although it is possible to measure tolerance as the increase in dose required to maintain a given level of intoxication, this has rarely been done for sedating drugs [but see (Okamoto et al. 1978)] and usually tolerance is indexed as the attenuation of the initial response (e.g., motor impairment, hypothermia, depression of rate of operant responding).

Functional tolerance can be further subdivided into three types based on the duration and/or frequency of alcohol exposure. Chronic tolerance is seen with multiple injections or other exposure regimens. It used to be thought that functional tolerance to alcohol took days or weeks of repeated or continuous exposure to develop (Kalant et al. 1971), but we now know that it can develop more quickly.

At the other extreme, acute functional tolerance was first reported by Mellanby (1919) who studied dogs walking on a treadmill while implanted with a jugular catheter. He infused alcohol and recorded the blood alcohol level at which the animals first began to stumble and drag their feet. After a period, he discontinued the infusion, and recorded another blood alcohol level when the animals first regained the ability to walk without stumbling. The recovery alcohol level was higher than the initial value, indicating that a higher dose was necessary to produce intoxication at the later time point, which suggests the existence of AFT (as no measures were taken of brain alcohol levels, metabolic tolerance could not be ruled out). AFT has since been demonstrated in mice by comparing blood alcohol levels at recovery and loss of function (ability to remain balanced) on a rod (Gehle and Erwin 2000). AFT is the type of tolerance apparently shown by Schuckit's FHP subjects, as it apparently occurs within a single alcohol dosing session.

Bridging the gap between AFT and chronic tolerance, mice (Crabbe et al. 1979) and rats (Khanna et al. 1991) have shown a third type of tolerance, rapid tolerance, where response to a second injection of alcohol is reduced from the initial response. While chronic and rapid tolerance appear to be similar mechanistically, this is less certain for AFT, which may represent a unique adaptation (Kalant 1998).

4.2 Withdrawal Severity

The occurrence of withdrawal symptoms when a drug is discontinued is interpreted to mean that a state of dependence on the drug was present (Kalant et al. 1971). While some suggest that physical and psychological dependence are distinguishable entities, we do not see how this distinction can easily be made. For alcohol dependence, it has long been known that a range of withdrawal symptoms appear with characteristic temporal waxing and waning severity (Victor and Adams 1953; Isbell et al. 1955). Alcohol withdrawal symptoms include irritability, nausea, vomiting, tremor, anxiety, insomnia, hyperthermia, hyperventilation, tachycardia, and central nervous system hyperexcitability manifested as convulsions, seizures, hallucinations, and delusions (Metten and Crabbe 1996). The core symptoms are remarkably conserved across species that have been studied with certain species-specific exceptions [e.g., rodents cannot vomit; hallucinations and delusions would be difficult to document in rodents; rodents show numerous behavioral symptoms that are not extensively documented in other species (Friedman 1980)]. As with tolerance, withdrawal can be acute or chronic. For humans, acute withdrawal usually refers to symptoms that occur early after drinking ceases, and later symptoms may be described as protracted withdrawal or abstinence (Heilig et al. 2010).

Assessing the DSM-IV-R criterion of withdrawal is typically done by asking a question such as the following after showing the respondent a page full of symptoms including “the shakes,” “trouble sleeping,” “feeling anxious,” “heart beating fast” etc.: After cutting down or stopping drinking did you ever experience any of these problems? Follow-up questions would then assess the number and duration of the withdrawal symptoms.

Alcohol dependence is typically induced in rodents using the liquid diet or vapor inhalation procedures described in the previous section. Occasionally multiple injections or intubations of the drug are given. For mice, the most frequently studied behavioral index of withdrawal severity is the handling-induced convulsion, or HIC (Goldstein and Pal 1971). This sign ranges from a mild myoclonus through clonic convulsions and if an animal is severely dependent, it may show lethal tonic hindlimb extensor seizures. Severity of withdrawal is a joint function of alcohol dose and duration of exposure (Goldstein 1972) and the symptoms normalize after a few days. This behavioral sign is very sensitive, and has allowed investigators to document an acute withdrawal reaction (increased convulsions) a few hours following a single high dose of ethanol (Crabbe et al. 1991). Rats do not exhibit handling induced convulsions (Heilig et al. 2010) and withdrawal severity is generally indexed by a collection of somatomotor and other behavioral and physiological disturbances (Majchrowicz 1975).

Recently, there has been a great deal of interest in the possibility that even weeks after alcohol withdrawal is initiated, behavioral signs of anxiety-like behavior may be detectable in rats (Valdez et al. 2002; Heilig et al. 2010;

Pandey et al. 1999; Wills et al. 2009). While anxiety-like behavior has been reported in mice early during ethanol withdrawal, it is more difficult to document unequivocally in mice and has rarely been studied weeks after withdrawal has been initiated (for review, see Kliethermes 2005).

5 Rodent Methods for Assessing Genetic Correlation

Most behavioral traits are influenced by many genes, and usually any single gene exerts a relatively small effect on the trait. This broad genetic influence reflects the underlying biology. For example, "alcohol tolerance" is not mediated by a single neurotransmitter system and does not result from changes in a single brain area or circuit. Thus, the extremely powerful tools for manipulating single genes, including production of null mutants, gene knockdowns, viral mediated gene transfer, and transgenic over expression of a gene are unlikely to help us understand whether the total collection of genes influencing two traits are highly correlated or mostly distinct. The fact that one gene affects two traits is insufficient evidence for overall shared genetic risk. For example, nearly 100 genes have been targeted to produce null mutants or over expression transgenics, and many of these mice have been tested for ethanol preference drinking. When these studies were reviewed, the results showed that 1/3 of the genes appeared to produce a modest increase in preference drinking, 1/3 a modest decrease, and 1/3 were without effect (Crabbe et al. 2006). Thus, we do not consider the studies involving targeted genes for our assessment of genetic correlation.

Two genetic methods allow a relatively powerful assessment of genetic correlation. The first is to selectively breed lines of rats or mice for one target trait. In this laboratory analog of natural selection, breeders are chosen from the extreme responders in the population, and over generations, the selected line develops an extreme response. Usually, a parallel line is selected for low response. The genetic mechanism at work in a successful selective breeding project is that the frequencies of alleles at genes that influence the trait under selection are increased until all animals have two copies of the same allele for each relevant gene. If the selected lines are now compared for the trait postulated to be genetically correlated, and are found to differ, the most likely explanation is that the second trait is a genetically correlated response to selection. The principal limitation of this approach is that only those traits that have been selected for can be assessed, but its strength is that the potential correlated responses that can be tested are unlimited. The many methodological intricacies and caveats surrounding this approach have been discussed elsewhere (Crabbe et al. 1990).

The other relatively powerful method is to use inbred strains. Within an inbred strain of mice or rats, close relatives have been mated for more than 20 generations. The result of this inbreeding resembles that of selective breeding—gene

frequencies increase and eventually become "fixed" and all animals possess two identical alleles (i.e., are obligate homozygotes) at each gene. There are however two major differences. The specific allele at each gene that is fixed in an inbred strain has no necessary relationship to any phenotype—it has been captured by chance. Second, unlike selected lines, inbred strains are homozygous for all genes (in selected lines, multiple alleles continue to segregate at all genes unrelated to the trait under selection). If inbred animals from a substantial number of strains are tested for two traits, their mean responses can be correlated to assess genetic correlation rather directly. The more strains that can be tested, the more powerful is the test of genetic correlation. Many studies have been performed with one specialized set of inbred strains called BXD recombinant inbred (BXD RI) strains. These resemble standard inbred strains except that they are originally derived from the intercross of C57BL/6J And DBA/2J inbred strains and therefore have a much simpler genetic structure. Only two alleles are possible at any gene, one derived from each progenitor inbred strain. Because these two progenitor inbred strains differ markedly in response to nearly all drugs of abuse, including alcohol, the BXD RI strains display a wide spectrum of responses to alcohol for nearly all traits. Their use for gene mapping has been described elsewhere (Palmer and Phillips 2002), but for the present discussion, they are a very similar tool for assessing genetic correlation of two traits. Technical details surrounding the inbred strain panel approach have been discussed elsewhere (Crabbe et al. 1990).

A final group of issues surrounds the nature of the human experiment. The human data discussed here were derived from monozygotic (identical) and dizygotic (fraternal) twins. Two individuals from an inbred mouse or rat strain are a plausible surrogate for one monozygotic twin pair (but not a perfect one—there is no heterozygosity within an inbred strain). But no dizygotic twin pair can be produced that shares the parental genetic background with an inbred strain. And although there are more than 100 available mouse standard inbred strains, and several sets of multiple RI strains, studying enough inbred strains to perform a path analysis like that presented in this article would present a host of logistical and financial challenges.

6 Evidence for Genetic Correlation Across AD Criteria in the Rodent Literature

We consider here in turn the evidence from selectively bred animal lines; from correlations among strain means for standard inbred mouse strains; and strain mean correlations from the BXD recombinant inbred strain panel. The traits studied are summarized in the *Sidebar*, and the correlations across strain means are given in Tables 1 and 2.

Table 1 Inbred strain correlations between alcohol tolerance and withdrawal phenotypes

	Trait	2	3	4	5	6	7	8	9	10
1	HT chronic 30-3	**0.85**	**0.91**	**0.49**	**0.71**	**0.66**	−0.16	0.10	−0.09	0.34
2	HT chronic 60-3		**0.74**	**0.56**	**0.63**	**0.75**	−0.23	0.02	−0.09	0.32
3	HT chronic 30-5			**0.63**	**0.89**	**0.83**	−0.04	0.12	−0.06	0.25
4	HT chronic 60-5				**0.52**	**0.60**	−0.12	−0.20	−0.05	0.16
5	HT chronic 30-8					**0.92**	0.18	−0.19	−0.25	−0.05
6	HT chronic 60-8						0.05	−0.23	−0.24	−0.06
7	AFT LORR							−0.31	0.07	**(−0.63)**
8	Acute WDR								**0.57**	0.38
9	Chronic cont. WDR									**0.59**
10	Chronic interm. WDR									

Correlations in bold, $P < 0.05$ ($P = 0.07$). Correlations are based on 8–20 strain means
Variables 1–6 are from Crabbe et al. (1982). "HT chronic" = hypothermic tolerance, indexed as change from baseline temperature. "30–3" indicates 30 min after baseline temperature on the 3rd injection day, "60–8" refers to 60 min after baseline on the 8th injection day, etc
"AFT-LORR" is the acute functional tolerance to the loss of righting reflex from Ponomarev and Crabbe (2004)
"Acute WDR" is the area under the withdrawal handling-induced convulsion curve after a single alcohol injection from Metten and Crabbe (1994)
"Chronic cont. WDR" is the area under the curve for HIC following 72 h continuous vapor inhalation from Metten and Crabbe (2005)
"Chronic interm. WDR" is the area under the HIC withdrawal curve following intermittent vapor exposure from Metten et al. (2010)

6.1 Selected Lines

Mouse lines have been selected for the severity of withdrawal HICs (Crabbe et al. 1985). Starting with a genetically heterogeneous stock of mice where as many as eight alleles were segregating for any locus, a large population of animals was exposed to ethanol vapor for 72 h to induce a state of physical dependence. After removal from the inhalation chambers, mice showed waxing and then waning HIC severity for up to 24 h, with peak HICs seen at about 7–10 h into withdrawal. Mice with the most severe withdrawal HICs were mated to produce the ensuing generation of Withdrawal Seizure-Prone (WSP) mice, and those with the least severe HICs were mated to initiate the Withdrawal Seizure-Resistant (WSR) line. The experiment is replicated, so there were two, genetically independent WSP (WSP-1 and -2) and WSR (WSR-1 and -2) pairs of lines generated. Each generation thereafter, each line was reproductively isolated, and the most (or least) severe-scoring mice were used as mating pairs.

By the 11th selected generation, both WSP lines had at least tenfold more severe withdrawal HICs than their respective WSR lines, and heritability of the trait was about $h^2 = 0.26$. Mice from these early generations of selection were tested for other signs of ethanol withdrawal and were found to differ in some

Table 2 BXD Recombinant Inbred strain correlations between alcohol tolerance and withdrawal phenotypes

	Trait	2	3	4	5	6	7	8
1	HT chronic 2 g/kg	−0.13	0.22	0.05	−0.31	0.31	0.23	0.11
2	HT chronic 3 g/kg		0.13	−0.26	0.14	−0.02	0.06	0.20
3	HT chronic 4 g/kg			−0.20	−0.03	−0.01	−0.08	0.13
4	Grid test				0.20	0.17	0.24	0.10
5	AFT Dowel (Kirstein)					0.23	0.06	**0.43**
6	AFT Dowel (Gallaher)						**(0.42)**	**0.58**
7	Acute WDR							**0.63**
8	Chronic cont. WDR							

Correlations in bold, $P < 0.05$ ($P = 0.06$). Correlations are based on 8–32 strain means
“HT Chronic” variables refer to the attenuation of the hypothermic response to the stated dose of ethanol after three daily injections, as described in Crabbe et al. (1994, 1996)
“Grid test” data are locomotor ataxia scores from Phillips et al. (1996)
“AFT Dowel” are the acute functional tolerance scores on the dowel test, using two different methods (Kirstein et al. 2002; Gallaher et al. 1996)
“Acute WDR” are handling-induced convulsion scores as described for Table 1. Data are from unpublished studies conducted by P. Metten and J. Belknap, with permission
“Chronic Cont. WDR” are as described in Table 1, from Crabbe 1998

e.g., tremor) but not all (e.g., reduced activity) other ethanol withdrawal signs (Kosobud and Crabbe 1986). WSP mice have more severe withdrawal HIC after acute or chronic treatment with numerous other sedative hypnotic compounds, and they also differ in a number of other behavioral and neuropharmacological features (for reviews, see Metten and Crabbe 1996; Finn et al. 2004).

Naive mice from selected generations 7–16 were tested for tolerance to the hypothermic effects of ethanol. Ethanol was given for 3 days at 3.5 g/kg ip, and the reduction in body temperature was measured. By the third day, significant chronic tolerance was seen, but there was no significant difference between WSP and WSR lines in the magnitude of tolerance. An addition experiment gave 3.5 g/kg ethanol twice daily for 5 days to increase the amount of tolerance that developed, but WSP and WSR mice still did not differ.

Separate groups of mice were tested for attenuation of the duration of the loss of righting reflex following three daily 4 g/kg injections. Neither selected line developed chronic tolerance. Ethanol was then given twice daily at 3.5 g/kg for three days, and loss of righting reflex duration tested on the fourth day after 4 g/kg (parallel groups received saline only on days 1–3). Both WSP and WSR mice developed significant tolerance, but to an equivalent extent.

In all the above experiments, mice of both replicates of the selected lines were tested, with equivalent outcomes. This greatly strengthens the interpretation of a lack of a genetic correlation between tolerance measures and chronic withdrawal severity, as apparently correlated responses to selection can arise by chance in the relatively small populations of mice maintained in long term selected lines if there

is only one pair of selected lines (Crabbe et al. 1990). For the hypothermic tolerance studies, blood ethanol concentration (BEC) assays confirmed that the tolerance was functional. In contrast, the fact that tolerant WSP and WSR mice regained righting reflex at the same BECs as those responding to their first alcohol injection indicated that the tolerance to loss of righting reflex in this experiment was pharmacokinetic.

Several other experiments have been performed to selectively breed mice for withdrawal severity. Some lines were made dependent using a liquid diet (Berta and Wilson 1992; Wilson et al. 1984) and others have used vapor inhalation (see Kosobud and Crabbe 1995). Yet others have been bred for the severity of acute withdrawal HIC (Metten et al. 1998). Unfortunately, none of these lines were ever tested for tolerance to any ethanol response, and all are extinct.

Mouse lines have also been selectively bred for two forms of ethanol tolerance. Starting with a segregating stock, two replicate pairs of mouse lines were selected for high (HAFT) or low (LAFT) acute functional tolerance to ethanol using a dowel balancing task (Erwin and Deitrich 1996). Mice were given an ip injection of 1.75 g/kg ethanol and repeatedly placed on a dowel beginning several minutes after injection until they could remain on the dowel without falling for 30 s. A blood ethanol sample (BEC_1) was taken. They were then injected with a dose of 2.0 g/kg and later tested again until they recovered ability to remain on the dowel. BEC_2 indexed this second recovery point. Acute functional tolerance (AFT) was defined as the difference in BECs (BEC_2-BEC_1).

High (HRT) and low (LRT) rapid tolerance mouse lines (in replicate) were selectively bred from a heterogeneous stock for a different tolerance phenotype (Rustay and Crabbe 2004). Mice were tested for two successive days for the effect of 2.5 g/kg ethanol to impair performance on an accelerating rotarod. The increase in latency to fall (Day 2−Day 1) was the selection index. These animals showed genetic differences in both rapid (two injection days) and chronic (five injection days) tolerance in this task.

Unfortunately, neither HAFT and LAFT mice nor HRT and LRT mice were ever tested for ethanol withdrawal severity, so the experiments that parallel those performed in WSP and WSR cannot easily be done. HRT and LRT are extinct. HAFT-2 and LAFT-2 mice are preserved cryogenically as embryos, so it would be feasible (albeit expensive) to resuscitate them for the purposes of testing for withdrawal severity.

In summary, the data from lines selectively bred for ethanol withdrawal severity differences suggest that genetic contributions to withdrawal and tolerance phenotypes are generally distinct.

6.2 Standard Inbred Strains

Several data sets have been published documenting mouse inbred strain differences in the severity of alcohol withdrawal. All have employed the HIC to index withdrawal severity. Fifteen inbred strains were studied for 24 h following a single

ip injection of 4 g/kg ethanol (Metten and Crabbe 1994). Eighteen strains were exposed to ethanol vapor inhalation for 72 h and found to differ in withdrawal severity (Crabbe et al. 1983). A limitation of this early study was that strains differed markedly in their BEC during inhalation, hence in the dose of ethanol to which they were chronically exposed. We do not consider those data here. This experiment was repeated more recently with 15 inbred strains using a procedure that exposed different strains to different ethanol vapor concentrations in order to match them for experienced dose. This experiment also yielded significant inbred strain differences in withdrawal severity independent of dose administered (Metten and Crabbe 2005). Finally, recent interest has emerged in studying ethanol withdrawal using a procedure where vapor exposure is limited to 16 h/day with 8 h exposure to air, for 3–4 days (Lopez and Becker 2005). Thirteen strains were characterized for withdrawal following this chronic intermittent exposure paradigm (Metten et al. 2010). Two data sets with a substantial number of inbred strains have reported ethanol tolerance magnitude. Eighteen strains were given 3.0 g/kg ethanol ip for 8 days and the reduction in body temperature was assessed at several times following injection on Days 1, 3, 5, and 8. Magnitude of chronic tolerance was indexed as the attenuation of Day 1 hypothermic response on each of Days 3, 5, and 8 (Crabbe et al. 1982). Twenty strains were assessed for the development of acute functional tolerance to the effect of a single dose of ethanol to induce loss of the righting reflex. Acute functional tolerance was indexed as the difference between BEC at regain and loss of righting reflex (Ponomarev and Crabbe 2004).

We examined the strain mean correlations for the three above withdrawal and seven (six hypothermia, one loss of righting reflex) tolerance phenotypes. Depending on strain overlap across studies, these correlations were based on between 8 and 20 strains (see Table 1). Of the 18 correlations between withdrawal and hypothermic tolerance scores, the largest absolute value was $r = 0.34$ ($df = 8$, NS). All correlations with withdrawal severity after chronic continuous exposure were negative, as were two of the six with withdrawal following chronic intermittent exposure. Correlations with acute withdrawal severity were evenly split between positive (after 3–5 days in the hypothermic tolerance regimen) and negative (after 5–8 days) data points. Acute functional tolerance to the loss of righting reflex tended to correlate significantly, and negatively ($r = -0.63$, $df = 7$, $P = 0.07$) with withdrawal from chronic intermittent vapor exposure, but not with acute ($r = -0.31$) or chronic continuous exposure ($r = 0.07$).

These data sets were constructed so that the tolerance measures represent functional tolerance, albeit of two sorts, acute and chronic, and were based on two different behavioral end points. The withdrawal measures also were controlled for ethanol dose experienced. Why was loss of righting reflex tolerance weakly associated with chronic intermittent and not chronic continuous or acute withdrawal phenotypes? This is because these three withdrawal phenotypes are themselves only imperfectly associated at the genetic level. Overall, the pattern of results suggests that there is no significant degree of overlap in the genetic contributions to withdrawal and tolerance phenotypes.

6.3 Recombinant Inbred Strains

Most recombinant inbred strain data relevant for alcohol genetics have been collected in the BXD RI strain panel. Twenty-one of these strains have been characterized for acute withdrawal severity after a single 4 g/kg ethanol dose (Metten and Belknap, unpublished data). They have also been exposed to continuous vapor inhalation for 72 h and scored for chronic withdrawal severity (Crabbe 1998). No data are available for these strains following chronic intermittent exposure, but these data are currently being collected (H. Becker, personal communication). Twenty-five strains have been tested for hypothermic tolerance to ethanol injections. The grid test was used to characterize the development of tolerance to ambulatory ataxia in 24 strains.

Two different groups assessed functional tolerance to ethanol's effects on a dowel balancing test. One experiment followed the exact procedure employed to breed the HAFT and LAFT selected lines described earlier (Erwin and Deitrich 1996) and found significant RI strain differences in acute functional tolerance (Kirstein et al. 2002). The other study followed a slightly different procedure (Gallaher et al. 1996—see *Sidebar*).

Genetic correlations across withdrawal and tolerance phenotypes measured in the BXD RI strains are shown in Table 2. As seen in the standard inbred strains, chronic hypothermic tolerance was not significantly correlated with any of the three withdrawal measures; nor was chronic tolerance in the grid test. There was, however, a pattern of significant correlation between acute tolerance in the dowel test and chronic withdrawal severity. These correlations accounted for 18 or 34% of the variance, depending on the tolerance assay. While acute withdrawal severity tended to be associated with the tolerance as measured by Gallaher et al. ($r = 0.42$, $df = 19$, $P = 0.06$), it was essentially uncorrelated with tolerance in the Kirstein procedure ($r = 0.06$). As with the standard inbreds, there was, therefore, a lack of complete parallelism of results between tolerance and different withdrawal measures. Again, this was likely because the two dowel test tolerance measures were themselves very weakly associated ($r = 0.23$), and the two withdrawal measures were imperfectly associated ($r = 0.63$).

6.4 Summary of Rodent Data

Data from rodents do not in our opinion offer strong and consistent evidence for a genetic relationship between the various tolerance and withdrawal phenotypes explored. The strongest evidence for such an association was seen in the BXD RI strains, where chronic withdrawal HIC severity after chronic continuous administration of ethanol vapor was significantly genetically correlated with tolerance assessed in two different variants of the dowel test. These two tolerance variants resemble AFT, but neither represents the classic version of this type of tolerance.

The importance of this relationship for answering to larger questions in human drinkers should be assessed in the context of several qualifications. First, the shared variance accounted for a relatively small proportion of the total variance (18 or 34%). Second, the relationship was only seen for acute withdrawal severity in one of the two data sets, and even there the correlation was small ($r = 0.42$). Third, the only genetic variance in these data sets arose from alleles polymorphic between C57BL/6J and DBA/2J inbred progenitor strains. Fourth, the only hint of a relationship in standard inbred strains, where there is substantially greater genetic diversity, was a trend toward a *negative* genetic correlation between AFT to a different behavioral endpoint and one, but not two other, measures of withdrawal severity. Finally, no evidence of consequential differences in several measures of tolerance to two different behavioral end points was seen between mice bred to have very large differences in acute and chronic withdrawal severity.

7 Consilience in Studies of Gene Expression

All the rodent studies reviewed above were designed to explore one source of genetic variation, due to allelic differences at genes, i.e., polymorphisms. The allelic differences were either chance occurrences (inbred strains) or engineered by affecting allelic structure through systematic selective breeding. Such genetic differences can be traced to differences in DNA sequence, and such polymorphisms appear in all cells, at all times. Another source of genetic variation is also important. Not all copies of each gene are constantly expressed. Gene expression leading to RNA and protein synthesis clearly differs across time including developmental course, and different genes show very different temporal patterns of expression. The same gene may show very different temporal patterns of expression in different brain areas. To understand genetic influences on AD risk we therefore need to consider brain-regional differences in the expression of genes and how they are affected by chronic ethanol.

As noted in the previous sections, the two rodent phenotypes that can be most closely related to human AD are withdrawal and tolerance. Unfortunately, neither of these phenotypes has been extensively studied from the perspective of global gene expression. In contrast, there are extensive gene expression data on preference drinking (see e.g. Mulligan et al. 2006; Tabakoff et al. 2009; McBride et al. 2010). With this point in mind, the discussion on the expression data has been expanded to include preference drinking, recognizing that this phenotype only imperfectly aligns with any aspect of the AD associated symptoms. Before reviewing the expression data, there are several issues that require comment.

The first issue is that the brain regions and circuits associated with alcohol-related phenotypes are still being defined. A role for the corticotropin releasing factor-rich central nucleus of the amygdala (CeA) in withdrawal and dependence phenotypes has been suggested (see Roberto et al. 2003; Koob and LeMoal 2005; Koob and Volkow 2010). Chen et al. (2008) found that the lateral aspect of the

substantia nigra pars reticulata is required to express the acute withdrawal HIC phenotype in mice; withdrawal from chronic ethanol exposure appears to involve a circuit associated with the CeA, the basolateral amygdale (BLA), the dentate gyrus, the CA3 region of the hippocampus, the lateral septum, and the prelimbic cortex (Chen et al. 2009). Withdrawal from chronic intermittent ethanol exposure appears to involve a very similar circuit (Oberbeck and Hitzemann, unpublished observations). To our knowledge, there are no similar studies focusing on the circuits associated with acute or chronic functional tolerance. For preference drinking it is generally assumed that some aspects of the brain's reward pathways are involved (see Koob and Volkow 2010). However, unlike the situation for stimulant drugs of abuse, 6-hydroxydopamine lesions of the nucleus accumbens (NAc) or the ventral tegmental area have been found in some but not all studies to have little effect on ethanol consumption (see e.g. Rassnick et al. 1993; Fahlke et al. 1994; Ikemoto et al. 1997). Moller et al. (1997) found that lesions of the CeA but not the BLA reduced ethanol consumption in rats. Dhaher et al. (2008, 2009) found that lesions of the CeA but not the lateral posterior portion of the bed nucleus of the stria terminalis or the medial shell region of the NAc reduce ethanol consumption in a limited access two-bottle choice paradigm.

The second and related issue is whether the brain regions associated with human AD have strict counterparts in the rodent brain. For example, Koob and Volkow (2010) emphasize the role(s) of prefrontal areas such as the human orbital prefrontal cortex which may have no equivalent in the rodent (see Price 2007). Peters et al. (2009 and references therein) have emphasized a rodent circuit associated with drug abuse that involves the ventromedial prefrontal cortex. Key regions are the infralimbic and prelimbic cortex. In the non-human primate and human brain, these regions most closely align with areas 25 and 32 and rostral aspects of the anterior cingulate. To our knowledge, there are no published studies that have attempted a cross-species comparison of global gene expression across "equivalent" cortical brain regions from mouse or rat to man. It may well be possible to align brain regions based on function rather than anatomical features but this needs to be done cautiously.

The third issue involves the microarray technology used to assess global gene expression. Over the past decade, improvements in both microarrays and analytical techniques have made it possible to measure changes in brain gene expression quite accurately; importantly, the cumulative data indicate that most of the changes associated with behavioral phenotypes are actually quite small and in the range of 15 to 30% (see Mulligan et al. 2006; Bice et al. 2006). To some extent, the small changes reflect the fact that the hybridization isotherms for oligonucleotide arrays are frequently not linear due to probe saturation (Pozhitkov et al. 2010). This is true for both rodent and human arrays; thus, small but significant changes in one species may drop below the threshold for detection in another species, especially given that sample sizes are frequently limited. A related problem that makes comparison across species difficult is the effect of single nucleotide polymorphisms (SNPs) on gene expression (e.g. Peirce et al. 2006; Walter et al. 2007, 2009). False positives and negatives in one species can be

difficult to align with another. SNP masking is one solution to this problem but this assumes that one knows most of the high frequency SNPs. A fourth problem with microarrays arises from the annotation and summarization issues associated with predefined reporters/probes (Allison et al. 2006). Annotation problems continue, despite continued improvements in sequence information. Thus, caution still must be exercised when comparing data on "gene X" across species. Furthermore, microarray technology provides limited information about alternative splicing, microRNAs and almost no information about other non-coding RNAs. The importance of the non-coding RNAs to the regulation of gene expression and to our understanding of complex traits has been summarized (Lander 2011). In this regard, the emergence of next-generation sequencing and the RNAseq application provides a clear alternative to microarrays for detecting differential gene expression and effectively deals with the problems noted above (see Mardis 2011).

7.1 Human Expression Data from Post Mortem Brain Tissue

The number of human *post-mortem* global gene expression studies is relatively small but there has been remarkable congruence among the studies (see Liu et al. 2006). To our knowledge Lewohl et al. (2000) were the first to use microarrays (cDNA arrays) to examine gene expression in tissue from alcoholics and matched controls (this also appears to be the first use of microarrays for any alcohol-related study). Although the number of samples and the number of genes interrogated were relatively small, these authors noted some marked differences between groups in the cortical expression of myelin-related genes. A follow-up study (Mayfield et al. 2002) using a larger cohort and an improved array confirmed differences in the expression of the myelin-related genes. Genes affected included myelin-associated glycoprotein, apolipoprotein D, glial fibrillary acidic protein and oligodendrocyte-myelin glycoprotein. An additional key finding was that a number of genes involved in protein trafficking were altered in the alcoholic case groups. Members of this group included genes involved in variety of functions such as vesicle docking, synaptogenesis, and synaptic plasticity. Liu et al. (2006) provided additional data from the same laboratory but also integrated the results across several different laboratories (e.g. Flatscher-Bader et al. 2005; Sokolov et al. 2003). Importantly, these authors identified 27 genes that were changed in alcoholics across multiple studies and included in this list were myelin-related genes e.g. proteolipid protein 1. The repeated observation of an effect on myelin-related genes aligns with a well-described alcoholic neuropathology (e.g. Harper and Kril 1990).

There appears to be only a single study that has investigated human global gene expression in a non-cortical area. Kryger and Wilce (2010) examined gene expression in the BLA. The sample included ten alcoholics and ten controls obtained from the New South Wales Tissue Resource Centre at the University of Sydney; samples from this same resource were used in some of the studies

described above. A large number of genes were found to be differentially expressed; 212 were up-regulated and 560 were down-regulated. It is impossible to summarize all of these findings here; rather, three main findings are emphasized. (a) There was marked reduction for the alcoholics in the expression of oxyreductases, including many genes associated with energy metabolism; these data are consistent with the results of positron emission tomography scans on alcoholics (Volkow et al. 1992). (b) Protein trafficking and vesicle docking genes showed abnormal expression, which aligns with results from other studies (see above). (c) Kryger and Wilce (2010) emphasize some marked changes in glutamate related genes including *GLAST*, *GLT-1* and *GluR2*. Western blots were used to confirm protein changes in these genes.

In summary, human *post-mortem* studies have detected some marked changes in gene expression between alcoholics and controls. Despite the many potential confounds, there are some consistent themes which can be linked to the known neuropathology. While such studies may relate to the presumed tolerance and withdrawal experience *ante mortem* by the alcoholic subjects, they cannot distinguish between them as potential drivers of the expression differences as compared with controls.

7.2 Rodent Expression Studies

Rodent studies by and large have taken a different approach to the problem. Rather than looking at the effects of chronic ethanol consumption or exposure, the focus has often been on integrating brain gene expression data with gene mapping analyses, i.e., the emphasis is on finding genes which predispose one to excessive ethanol consumption or pronounced withdrawal symptoms. There have been extensive studies using informative genetic populations to identify the genomic location of quantitative trait loci (QTL). QTL mapping studies have isolated the chromosomal loci associated with many alcohol-related traits, and expression analyses have been employed to seek candidate genes for these QTL. This difference in approach, termed "genetical genomics," is an obvious barrier to achieving consilience between the rodent and human data sets as the approaches differ tactically.

The gene encoding multiple PDZ domain protein (MPDZ), which has been identified as an alcoholism-related gene in human genetic association studies, has been found in mice to be a quantitative trait gene associated with acute ethanol withdrawal HIC severity (Shirley et al. 2004). Ethanol tolerance, the other consilience phenotype, has been less well studied. Hu et al. (2008) used the genetical genomic approach to identify eight genes associated with AFT. Interestingly, one of these genes, erythrocyte membrane protein band 4.1-like 2 (*EPB41l2, or 4.1G*) is a cytoskeletal protein that interacts with AMPA receptor GluR1 and GluR4 subunits, and may support their surface expression (Coleman et al. 2003). Bell et al. 2009 examined the effects of chronic ethanol exposure in the alcohol

preferring P rats on gene expression in the nucleus accumbens; neither the degree of tolerance that may have developed nor whether dependence had developed were assessed. Two different paradigms were used to administer ethanol chronically: (a) multiple scheduled access (three, 1-hr, dark-cycle sessions/day) for 8 weeks; and (b) continuous, daily alcohol access (24 h/day). The control group was ethanol na. Average daily ethanol intakes for the continuous and multiple groups were approximately 9.5 and 6.5 g/kg/day. Animals were sacrificed 15 h after the last ethanol exposure: assuming that the animals were dependent, the animals were in the early stages of withdrawal. Interestingly, the multiple access group showed few changes in gene expression. In contrast, 374 genes were detected as significantly different between the continuous access and ethanol-na groups. Twenty significant Gene Ontology (GO) categories were over-represented and these included negative regulation of protein kinase activity, antiapoptosis, and regulation of G-protein-coupled receptor signaling. Some of the differentially expressed genes were ones that had been detected in human *post mortem* studies, e.g., *Scg2* (Mayfield et al. 2002).

Gene expression profiling has been used to study ethanol withdrawal severity in the WSP and WSR selected lines of mice (Hashimoto and Wiren 2008). Mice of both WSP and both WSR lines were exposed to equal ethanol vapor concentrations for 72 h. Eight hours into withdrawal, when HICs would have been marked in the WSP mice (and virtually absent in the WSR mice), the prefrontal cortex was harvested for microarray analyses. Mice were not scored for withdrawal, however. Mice of both sexes were tested. Ethanol withdrawal clearly regulated the expression of approximately 300 genes. Interestingly, there were large sex differences in the pathways identified by gene ontology overrepresentation analyses. These, and pathological analyses, were consistent with greater ethanol neurotoxicity experienced by female mice. However, there were no important differences between the WSP and WSR mice in the GO categories. Thus, while the genes and gene pathways identified clearly were ethanol withdrawal-responsive, they were not germane to the issue of the large genetic differences in ethanol withdrawal severity between WSP and WSR mice (Hashimoto and Wiren 2008). Another project examined cingulate cortex and amygdala tissue in Wistar rats after a long period of ethanol exposure (Rimondini et al. 2002). Rats were exposed to ethanol vapor for 8 weeks and then drank voluntarily for several weeks. Expression analyses of tissue harvested after recovery revealed several genes and pathways to be chronically up-regulated. This study and several others have been recently reviewed (Bjork et al. 2010).

It should be noted that at least among mice, there is an inverse genetic relationship between ethanol preference and severity of withdrawal HIC (Metten et al. 1998; Hitzemann et al. 2009). Thus, some candidate genes for preference phenotypes, and their associated gene networks, are highly likely to be involved in withdrawal, one of the target phenotypes for this discussion. Sandberg et al. (2000) appear to have been the first to integrate behavioral QTL and gene expression data. Three symposia reports (Hoffman et al. 2003; Matthews et al. 2005; Sikela et al. 2006) illustrate the application of this approach to alcohol related phenotypes,

which were mostly based on ethanol preference drinking. Mulligan et al. (2006) performed a meta-analysis using microarray data from six different samples of alcohol preferring and non-preferring animals. The data from a total of 107 arrays were entered into the analysis. The statistical power of the analysis allowed the authors to detect 3,800 genes uniquely and significantly changed between preferring and non-preferring animals. Several functional groups, including mitogen-activated protein kinase signaling and transcription regulation pathways, were found to be significantly over-represented. Focusing on the genes within the mouse chromosome 9 QTL for ethanol preference which has been detected in multiple studies (Belknap and Atkins 2001), several genes were detected as being highly differentially expressed between preferring and non-preferring animals; genes included in the group are *Scn4b, Scd5* and a number of genes with unknown function. *Scn4b,* which reduces ethanol effects on sodium channels, is currently under investigation using transgenic and viral mediated transfer strategies (Hitzemann—unpublished observations).

Tabakoff et al. (2008) used a somewhat different approach to detect genes associated with ethanol preference; however, *Scn4b* still emerged as a strong candidate. Importantly, this group has consistently found that *Gnb1* is differentially expressed between preferring and non-preferring animals. This group extended this observation and also summarized the genes in mouse, rat and humans that have been associated with excessive ethanol consumption (see Table 2 in Saba et al. 2011). These authors concluded that the activity of the GABAergic system, and in particular GABA release and GABA receptor trafficking and signaling including G protein function, contributes significantly to genetic variation in the predisposition to varying levels of alcohol consumption. This conclusion aligns with the known mechanisms of ethanol action (Spanagel 2009).

7.3 *An Alternative Strategy for Relating Gene Expression to Function*

The human and rodent gene expression studies outlined here all largely used the same data analysis tactic which focused on finding genes that are differentially expressed and then aligning these genes with known protein–protein interaction pathways. There are alternative analysis strategies. For example, the covariance structure of the gene expression data can be analyzed. One such tactic is the weighted gene co-variance network analysis (WGCNA) (Zhang and Horvath 2005). The advantages of this approach over looking at differential expression are discussed in Zhao et al. (2010). Here, we simply note that the focus is on looking at gene connectivity both within and between gene expression modules which may or may not be associated with differential expression. The disadvantage of this approach is the requirement for large sample sizes (see Iancu et al. 2010). Oldham et al. (2006) used WGCNA to examine the conservation and evolution of gene coexpression networks in human and chimpanzee brains. The data obtained illustrated two

important points. The first is that in both the human and chimpanzee brains, modules that correspond to brain regions would not be successfully detected simply on the basis of differential gene expression among brain regions. The second point is that the coexpression analysis led to the observation that the "[degree of] conservation of gene coexpression modules between the species recapitulates evolutionary hierarchy, with white matter > cerebellum > caudate nucleus > caudate nucleus + anterior cingulated cortex > cortex, again a relationship not evident from differential expression analysis." In a second study, Oldham et al. (2008) used WGCNA to examine the functional organization of the human brain transcriptome. Here, the coexpression analysis was able to parse the microarray data to identify different modules of coexpressed genes that corresponded to neurons, oligodendrocytes, astrocytes and microglia. Importantly, it was possible to place a number of genes of unknown function into one of these modules. As larger alcohol-related data sets for both human and rodent data evolve, it is not unreasonable to expect that it will be possible to use strategies such as the WGCNA to detect cross-species consilience in the gene expression data.

8 Conclusions and Future Directions

For the only human AD diagnostic criteria for which there are substantial rodent data, alcohol withdrawal and tolerance, those data largely support their genetic independence. Albeit quite limited in extent, nonetheless, we do observe a limited consilience between rodents and humans in this regard—that the genetic risk factors for alcohol-related tolerance and withdrawal are largely uncorrelated. This is true despite the close relationship between tolerance and dependence as pharmacological characteristics of chronic drug administration. That is, it is not likely to find an animal that is dependent but not tolerant.

For humans, many problems ensue in individuals as a result of their heavy drinking including work-related issues, fraying or disintegration of family relationships, psychiatric problems such as anxiety or depression and legal difficulties. Of these, only anxiety-like and depressive-like behaviors are modeled in rodents. Humans continue to drink despite increasing evidence of adverse medical or social consequences—indeed, some alcoholics effectively drink themselves to death. As discussed in Sect. 1, rodents do not drink enough alcohol under most circumstances to develop the sorts of major medical complications seen in humans with AD. However, one approach has recently been suggested to represent drinking despite adverse consequences. When rats or mice have been led to drink chronically under certain conditions, subsequent adulteration of the alcohol solution with quinine does not have the expected effect of reducing consumption as strongly as in animals with less experience drinking alcohol. Because quinine is normally aversive, this persistent drinking in the face of a normally punishing event may be considered to reflect, at least partially, drinking despite adverse consequences (Lesscher et al. 2010). However, such studies to date have not shown *why*

ethanol-experienced animals fail to avoid ethanol + quinine. Persistent drinking of an adulterated ethanol solution could simply reflect a change in the salience of the taste of quinine; we recently reported a rather direct example of carryover effects of alcohol solutions that abolished the subsequent taste preference for sweet solutions and the avoidance of quinine solutions (Crabbe et al. 2011).

Several approaches may begin to address the "loss of control" over drinking. When alcohol is offered in a standard two-bottle preference test with water as an alternative, it was noted many years ago that following a period of abstinence, ethanol preference drinking is escalated for a time after the re-introduction of access. This has been termed the alcohol deprivation effect, or ADE (Sinclair and Senter 1967). A recent variant of this procedure produces a robust increase in alcohol drinking in C57BL/6J mice (Melendez et al. 2006; Melendez 2011). However, the elevated drinking does not persist for very long, and no blood alcohol levels were taken in these experiments, so whether the animals reach intoxicating blood alcohol levels is not known. And, there are no published data currently available regarding genetic differences in this type of drinking. Scheduling access to fluids for a limited period each day leads C57BL/6J mice to drink large amounts and reach intoxicating blood ethanol levels (Finn et al. 2005; Cronise et al. 2005). A possibly related method is to offer ethanol to animals intermittently, either every other day (Wise 1973) or 3 days\week (Simms et al. 2008), but these animals do not reach intoxicating blood alcohol levels. Cunningham and collaborators have exposed mice to ethanol chronically via indwelling gastric cannulae. Mice thereafter are willing to self-administer ethanol via intragastric infusion, and even a normally alcohol-avoiding genotype, DBA/2J, will self-administer substantial doses of alcohol (Fidler et al. 2011). We have bred high drinking in the dark (HDID) mice for binge-like drinking. These mouse lines drink to the point of behavioral intoxication, but have not been studied for tolerance or withdrawal traits (Crabbe et al. 2009). With the exception of the HDID selection, these phenotypes have yet to be genetically characterized extensively enough to address the questions at hand regarding human diagnostic criteria, but they may provide useful tools for future translational studies.

There are other data that would be very helpful if they were available. The tolerance mouse lines, HAFT and LAFT, now exist only in cryopreserved embryos, but could be resurrected and tested for withdrawal severity. This would offer a rather direct test of the genetic correlation between withdrawal and tolerance. The HRT and LRT mouse lines, selected for rapid (chronic) tolerance, would also be useful, but were not cryopreserved. A thorough characterization of the WSP and WSR selected lines for a range of ethanol tolerance phenotypes would be informative.

The existing data on gene expression differences, either predisposing to alcohol responses or consequent to exposure to alcohol, have unfortunately rarely characterized either tolerance or withdrawal phenotypes. A systematic characterization of the gene expression networks predisposing to and invoked by ethanol tolerance could be compared with the networks seen after initiating dependence, and during withdrawal. A maximally informative line of mice for such a genetic experiment would be the HS/CC outbred stock, which was developed by members of the

Collaborative Cross consortium specifically to display maximal allelic diversity (Churchill et al. 2004).

These results have obvious implication for efforts in human populations to study genetic risk factors for AD. In twin or adoption studies, which assess aggregate risk factors across the genome, prior studies that have looked at the magnitude of genetic effects, developmental processes or patterns of comorbidity have consistently assessed AD as if it reflected a single dimension of genetic liability. These results will need to be reconsidered in light of evidence for multiple genetic factors underlying AD. Molecular genetic studies—particularly candidate gene and genome-wide association studies—have similarly focused almost exclusively on the comparison of subjects meeting criteria for AD with matched controls. If correct, the results reviewed herein suggest that this approach would be at inefficient at best. Cases and controls would likely differ on three relatively independent dimensions of genetic risk with the degree of difference varying considerably across individuals. While these results need replication before they should lead to widespread changes in analytic strategy, they highlight the assumptions widely accepted but rarely tested that psychiatric and substance use disorders as described by current diagnostic systems reflect a single dimension of genetic risk. This assumption is unwarranted and should not be accepted prior to being subject to empirical test.

In summary, further refinements in both the human and rodent laboratory data are needed to determine whether AD represents one or multiple genetic factors. With the growing power of genetic analyses, it should be possible to improve our insight into human etiology, even if it is not possible to resolve completely the specific issue reviewed here.

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A.1 Appendix

Sidebar. Mouse measures of tolerance and withdrawal severity in Tables 1 and 2

Trait	Description	Tabled variables	Reference
Hypothermic tolerance (standard inbred strains)	Mice were injected daily for 8 days with 3.0 g/kg EtOH. Initial hypothermic sensitivity was indexed as difference scores, each representing the reduction (on day 1) from baseline at 30 or 60 min after injection. Tolerance on days 3, 5, and 8 was indexed as the difference in post-injection change score from day 1 sensitivity score	Table 1, traits 1 and 2 tolerance on day 3 (e.g., HT chronic 30–3 and HT chronic 60–3) Table 1, traits 3 and 4, tolerance on day 5 Table 1, traits 5 and 6, tolerance on day 8	Crabbe et al. (1982)

(continued)

(continued)

Trait	Description	Tabled variables	Reference
Acute functional tolerance Loss of righting reflex (standard inbred strains)	Mice were injected with 3.0 g/kg EtOH. Blood samples were taken when they lost the righting reflex (i.e., were unable to turn over from a supine position) and when they regained it. The difference in blood EtOH concentrations (recovery minus initial loss) indexed AFT	Table 1, trait 7 AFT LORR	Ponomarev and Crabbe (2004)
Acute withdrawal (standard inbred strains)	Mice were injected with 4.0 g/kg EtOH and the handling-induced convulsion (HIC) was scored before, and hourly after for 12 h. Withdrawal severity was indexed as the area under the HIC curve corrected for baseline HIC	Table 1, trait 8 Acute WDR	Metten and Crabbe (1994)
Chronic withdrawal—continuous (standard inbred strains)	Mice were continuously exposed to EtOH vapor for 72 h at an average blood EtOH concentration of 1.6 mg/ml. Withdrawal HIC severity was assessed hourly for 10 h and again at 24 and 25 h. The average area under the 25 h HIC withdrawal curve for each strain was corrected by subtracting the area for HIC scores from a group exposed to air	Table 1, trait 9 Chronic Cont. WDR	Metten and Crabbe (2005)
Chronic withdrawal—intermittent (Standard inbred strains)	Mice were exposed to EtOH vapor for 16 h/day for 3 days at an average blood EtOH concentration of 1.7 mg/ml. Withdrawal HIC severity was assessed hourly for 10 h and again at 24 and 25 h. The average area under the 25 h HIC withdrawal curve for each strain was corrected by subtracting the area for HIC scores from a group exposed to air	Table 1, trait 10, Chronic Interm. WDR	Metten et al. (2010)

(continued)

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Trait	Description	Tabled variables	Reference
Hypothermic tolerance (BXD RI recombinant inbred strains)	Mice were injected daily for 3 days with 2.0, 3.0 or 4.0 g/kg EtOH. Initial hypothermic sensitivity was indexed as the average difference from baseline at 30 and 60 min after injection on day 1. Tolerance on days 3, 5, and 8 was indexed as the difference in post-injection change score from day 1 sensitivity scores	Table 2, traits 1–3 HT chronic 2 g/kg, HT chronic 3 g/kg, and HT chronic 4 g/kg	Crabbe et al. (1994, 1996)
Grid test tolerance (BXD RI recombinant inbred strains)	Mice were injected with saline for two days, and EtOH 2.0 g/kg on days 3,5,7,9, and 11. The grid test was used to assess foot fall errors through a wire mesh floor on each EtOH day, corrected for locomotion. Tolerance was indexed as the difference between ataxia ratios (foot falls/activity) on days 11 and 3	Table 2, trait 4 Grid test	Phillips et al. (1996)
Acute functional tolerance Dowel test (BXD RI recombinant inbred strains)	Mice were injected with 1.75 g/kg EtOH and placed on a stationary, 1.27 cm dowel, from which they soon fell. A blood sample was collected when they recovered ability to stay on the dowel (BEC1), and they were given a second, 2.0 g/kg injection. Another blood sample (BEC2) was taken when they again regained ability. AFT was indexed as BEC2 minus BEC1	Table 2, trait 5 AFT Dowel (Kirstein)	Kirstein et al. (2002)
Acute functional tolerance Dowel test (BXD RI recombinant inbred strains)	Mice were given an injection of 2.0 g/kg EtOH. Brain EtOH levels were taken within 10 s of fall from a rotating, 5 cm dowel to assess initial sensitivity. Separate groups of mice were given the initial 2.0 g/kg injection, and when	Table 2, trait 6 AFT Dowel (Gallaher)	Gallaher et al. (1996)

(continued)

(continued)

Trait	Description	Tabled variables	Reference
	they recovered ability to stay on the dowel, a blood sample was taken. Mice were then given a "booster" dose of 1.0 g/kg and a second recovery was assessed. Four to five booster doses were given until each RI strain of mice was recovering function at a stable plateau of blood EtOH concentrations. The difference between the final and the initial brain EtOH concentration was taken as the index of tolerance		
Acute withdrawal (BXD RI recombinant inbred strains)	Same as for standard inbreds	Table 2, trait 7 Acute WDR	P. Metten and J.K. Belknap, unpublished data, with permission
Chronic withdrawal—continuous (BXD RI recombinant inbred strains)	Same as for standard inbreds. Average blood EtOH concentration was 1.5 mg/ml	Table 2, trait 8 Chronic cont. WDR	Crabbe (1998)

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Non-Human Primate Models of Alcohol-Related Phenotypes: The Influence of Genetic and Environmental Factors

Christina S. Barr

Abstract Because of their complex social structures, behaviors, and genetic similarities to humans, nonhuman primates are useful for studying how genetic factors influence alcohol consumption. The neurobiological systems that influence addiction vulnerability may do so by acting on alcohol response, reward pathways, behavioral dyscontrol, and vulnerability to stress and anxiety. Rhesus macaques show individual differences in alcohol response and temperament, and such differences are influenced by genetic variants that are similar functionally to those present in humans. Genes at which variation moderates these phenotypes include those encoding monoamine oxidase A (MAOA-LPR), the serotonin transporter (HTTLPR), corticotropin releasing hormone (CRH-248C/T and -2232 C/G), Neuropeptide Y (NPY-1002 T/G), and the μ-opioid receptor (OPRM1 C77G). These provide opportunities for modeling how genetic and environmental factors (i.e., stress, individual's sex, or alcohol exposure) interact to influence alcohol consumption. Studies in primates may also reveal selective factors have driven maintenance or fixation of alleles that increase risk for alcohol use disorders in modern humans.

Keywords Genetic · Gene-Environment · Stress · Macaque · Non-human primate · Alcohol

C. S. Barr (✉)
Section of Comparative Behavioral Genomics, NIH/NIAAA/LNG,
Rockville, MD 20852, USA
e-mail: cbarr@mail.nih.gov

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Contents

1 Introduction 224
2 Primate Models 225
3 Genetics of Temperament 226
4 Rhesus Macaque Candidate Gene Studies 227
4.1 Reward Drinking 227
4.2 "Relief Drinking": Gene × Stress Interactions 229
5 Gene × Alcohol Interactions and Addiction Vulnerability 234
5.1 Pharmacogenetics of Alcohol Response 234
5.2 G × E Interactions and Allostasis 235
6 Role of Genetic Selection: Understanding the Origins of Alcohol Use Disorders in Modern Humans 237
7 Sexually Dichotomous G × E Interactions 240
8 Summary 241
References 242

1 Introduction

Alcoholism and alcohol abuse are chronic, relapsing, lifetime illnesses that are notoriously difficult to treat. Although they are complex disorders, with multiple subtypes and clinical pictures, one defining feature is the chronic, maladaptive use of alcohol, which leads to compulsive ethanol seeking, often in the face of negative social and psychological consequences. Alcohol induces neuroadaptative changes, which result in allostatic shifts in both affect and reward threshold (Koob and Le Moal 1997, 2001). Animal models are critical to furthering our understanding of these neuroadaptive processes, and insight into the genetic and environmental factors that contribute to variability in adaptation to alcohol and the manner in which it relates to vulnerability, progression and pathogenesis of alcohol use disorders can also be accomplished with the use of animal models (reviewed in Lovinger and Crabbe 2005).

Various animal models have been effective for examining alcohol response (pharmacokinetics, tolerance, motor impairment, etc.), characterizing patterns of consumption, and examining the pathological effects of alcohol in brain (reviewed in: Barr and Goldman 2006; Heilig and Koob 2007; Lovinger and Crabbe 2005). However, non-primate models are limited by several factors, including shorter early developmental and adolescent time courses and differences in key systems the mediate stress response some of which are involved in the neuroadaptive changes that drive the development of alcohol dependence (i.e., CRH) (Sanchez et al. 1999). Nonhuman primates have an extended period of early and adolescent development and are similar to humans in their neuroanatomy, neurobiology, behavior and social organization. Furthermore, the genetic similarities among humans, apes and monkeys (especially Old World species) make them valuable for modeling how genetic factors increase risk for alcohol dependence. As a

consequence of these shared characteristics between nonhuman primates and humans, paradigms that use them to model aspects of human alcohol abuse and dependence provide a high degree of face validity (Tabakoff and Hoffman 2000).

2 Primate Models

Because of the benefit of gravitating to and selecting calorically-enriched, ripened plants, a number of different animal species will ingest alcohol containing fermented fruits (Dudley 2002). There are popularized tales of elephants and baboons stumbling across the Serengeti, intoxicated from the ingestion of fermented Marula fruit, of inebriated Howler monkeys in the forests of Panama jumping from tree to tree like drunken teenagers in order to feast on the fermented fruits of the Astrocarym palm, and even of vervets having stolen coconuts filled with rum from shipping docks in St. Kitts and drinking themselves into stupor.

In the laboratory, primates will self-administer ethanol. The Old World monkeys—vervets, or African Green Monkeys (*C. aethiops*), macaques (*M. mulatta*, *M. nemestrina*, or *M. fascicularis*) and baboons (Papio spp)—are the most popular models of study. Various routes of administration, dosing schedules, experimental regimens, and environmental manipulations have been demonstrated to induce oral alcohol consumption in nonhuman primates. Among these are the use of a palatable sweetened vehicle, food and water deprivation, schedule-induced polydipsia, and exposure to acute or to early life stress. Intragastric, intravenous and oral routes of administration have all been employed in nonhuman primate alcohol studies. Some of these routes of administration can result in consistently high blood alcohol concentrations and, as such, are very useful for examining pathological consequences of alcohol exposure (Grant and Bennett 2003; Meisch and Stewart 1994).

When attempting to model how genetic or environmental factors drive alcohol preference, rather than pathology, the method with greatest face validity is that of voluntary oral self-administration, since humans do not generally administer alcohol intravenously or by other methods. The taste of alcohol in these studies may function as both a conditioned reinforcer and a discriminative stimulus. And although rodent studies showing that passive exposure of alcohol is effective in modeling how repeated cycles of intoxication and withdrawal lead to dependence, these types of experiments are difficult to perform in primates. Studies investigating the effects of early adolescent exposure and intermittent alcohol access are in progress, and there are data suggesting that such approaches may be useful for modeling how genetic or environmental factors influence neuroplastic changes that underlie the transition from casual use to alcohol abuse or dependence in humans (Barr et al. 2004a; Lindell et al. 2010). Most studies examining effects of genetic variation on patterns of alcohol consumption in primates have focused on functional genetic variation that predicts behavior, alcohol response or preference in non-addicted subjects as a means of identifying genetic risk factors for the

development of alcohol problems (Barr and Goldman 2006). These studies are reviewed below.

3 Genetics of Temperament

The diversity of behavior as it relates to genetic variation in nonhuman primates has translational value in alcohol research. Certain temperament traits (i.e., novelty-seeking/impulsivity, reward dependence, and harm avoidance) can lead to human alcohol problems. Individuals who are anxious and routinely consume alcohol for its anxiolytic effects are predisposed to alcohol use disorders ("relief drinkers"), as are those who are impulsive, more likely to consume alcohol at an early age, and experience alcohol-related problems ("reward drinkers"). Although alcohol consumption can itself cause behavioral disinhibition and repeated alcohol exposures increase anxiety and affective disturbances, these temperament characteristics and alcoholism vulnerability are likely to involve common neurobiological and genetic susceptibility factors. Some of these traits may be influenced by neurobiological systems (i.e., dopamine or serotonin) that also influence alcohol response (see Sect. 5.1).

Individual differences in temperament have been extensively studied in a variety of nonhuman primate species. Early studies of personality differences in nonhuman primates indicated that rhesus macaques could be classified as being either "uptight" or "laid-back" (Suomi 1982; Higley and Suomi 1989). Vervet monkeys can be similarly characterized on the basis of behavior in the social group and responses to challenge (Bradwejn et al. 1992). In the social group, calm vervets are more active, are groomed more often and compete more effectively for resources. Uptight monkeys, on the other hand, are more isolated, and exhibit extremely submissive behaviors (cowering or crouching). When placed in a single cage, the uptight vervets exhibit stereotypical behaviors, whereas those that are laid-back sit quietly, exploring their environment. Using a paradigm to measure impulsivity, it has also been shown that there are marked individual differences in aggression and social impulsivity among vervets (Fairbanks et al. 2004).

Recent meta-analyses indicate that three personality dimensions are universally and reliably detected in nonhuman primates: Sociability, Confidence, and Excitability (Freeman and Gosling 2010), which have also been labeled as Gregariousness, Impulsivity, and Anxiety, respectively (Capitanio and Widaman 2005). Anxious (excitable) macaques exhibit increased behavioral and endocrine responses to stress in the laboratory. Field studies show that, in addition to being inhibited, excitable animals have higher cerebrospinal fluid levels of corticotropin-releasing hormone (CRH) and a high degree of EEG laterality (Kalin et al. 2000; Kalin and Shelton 2003), both of which have been documented in anxious or depressed human subjects. Other field studies show that impulsive (confident) macaques engage in risky behaviors and aggressive encounters and migrate from their natal troops at a younger age (Mehlman et al. 1994, 1995). They also have

lower cerebrospinal fluid levels of the serotonin metabolite, 5-hydroxy-indole-acetic acid (5-HIAA), which also occurs in Type 2, impulsive alcoholics (Westergaard et al. 2003). In both instances, these traits appear to be fairly consistent across time and situation (Higley et al. 1996a; Shannon et al. 2005; Kalin and Shelton 2003). Tools aimed at assessing behavioral responses to various stimuli (rather than looking only at temperament ratings) may be useful for accessing other dimensions that are relevant to the addictive disorders (Barr et al. 2008a).

As stated above, stress reactivity, novelty-seeking, and impulsivity are traits that may increase addiction vulnerability. Using a tool that assesses individual responses to an unfamiliar conspecific, studies have shown that both anxious and impulsive behaviors appear to be heritable in vervet monkeys (Fairbanks et al. 2004). Other studies show anxiety to be heritable in rhesus (Williamson et al. 2003). Although "uptight" vervets typically avoid alcohol in both social and individual cage testing paradigms (Palmour et al.1997; Ervin et al. 1990), laboratory studies show that anxious and impulsive rhesus macaques will consume more alcohol when tested under certain environmental conditions (Higley et al. 1991, 1996a, b). As "reward" and "relief" drinkers may seek alcohol in varied environmental contexts, the nonhuman primate model offers the potential for assessing how genetic factors relate to alcohol consumption using a controlled experimental system. Such findings may be particularly useful for accessing how genetic factors could contribute to vulnerability to a specific subtype of alcoholism (i.e., types 1 vs. 2, Cloninger 1987). The fact that alcohol consumption in outbred populations of rhesus macaques is heritable (Lorenz et al. 2006) lends further support to the appropriateness of the use of this macaque species for modeling human alcohol use disorders.

4 Rhesus Macaque Candidate Gene Studies

4.1 Reward Drinking

4.1.1 Monoamine Oxidase A (*MAOALPR*)

One gene at which variation is linked to impulsivity and impulsive aggression in both animal models and in humans is the monoamine oxidase A (MAOA) gene. MAOA degrades the monoamine transmitters (dopamine, norepinephrine, and serotonin), and, therefore can influence synaptic concentrations of these neurotransmitters. A variable number of tandem repeats (VNTR) polymorphism in the transcriptional control region for the human MAOA gene (MAOA-LPR) has been shown to produce differential transcriptional activity in vitro (Sabol et al.1998). In humans, the MAOA-LPR low activity alleles predict decreased prefrontocortical and increased amygdalar responses to emotional stimuli, suggesting impaired ability to control emotional responses during arousal (Meyer-Lindenberg et al. 2006). The low activity MAOA-LPR allele has also been associated with trait-like,

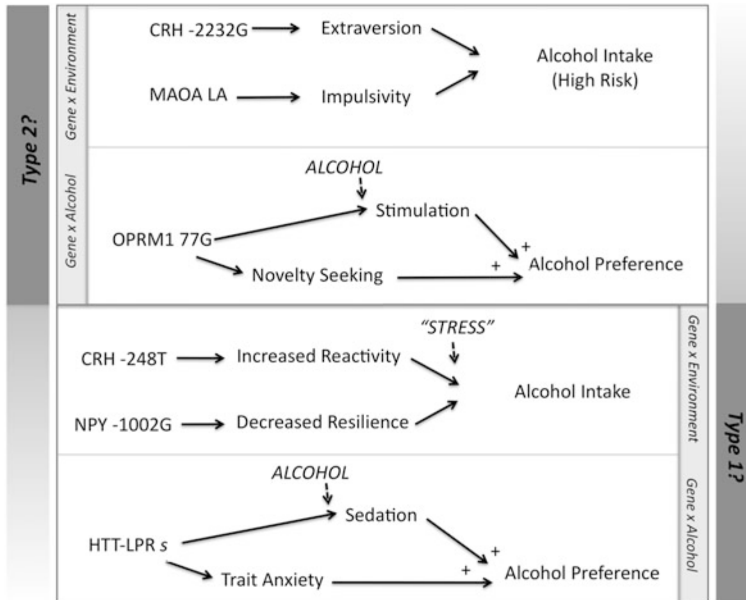

Fig. 1 Macaque studies suggest mechanisms by which genetic variation could increase alcohol use in humans. Functional genetic variation in macaques influences alcohol consumption through the pathways of decreased behavioral inhibition and enhanced stress reactivity (*gene* × *environment*). Macaque studies suggest that genetic factors that influence alcohol response and temperament may exert influences on alcohol consumption at multiple, interactive levels (*gene* × *alcohol*). These variants drive alcohol drinking in specific environmental contexts and may be useful for modeling how human functional genetic variation could increase the risk of alcohol use or abuse and make individuals especially prone to developing alcohol problems and moderate risk for types 1 or 2 alcohol dependence

alcohol-independent antisocial behavior in alcohol-dependent populations (Samochowiec et al. 1999; Tikkanen et al. 2009), and various studies have shown that early environmental factors interact with genotype to predict antisocial behavior, aggressiveness, and violence (Caspi et al. 2002; Sjoberg et al. 2008), traits that are present in early-onset, Type 2 alcoholics. Other studies show that MAOA genotype is associated with alcohol dependence, age of onset of dependence, and comorbid drug abuse (Contini et al. 2006).

The low activity allele of rhMAOA-LPR increases impulsivity among rhesus macaques (Newman et al. 2005; Wendland et al. 2006), and is also associated with increased alcohol consumption (Fig. 1). Among adolescent and young adult male rhesus macaques, the rhMAOA-LPR genotype accounts for approximately 10% of the variance in alcohol consumption (Barr et al. 2004d). These studies in rhesus macaques support the notion that MAOA gene promoter variation may specifically increase the risk for Type 2 alcoholism in humans, a subtype that is early in onset, highly heritable and more common among men.

4.1.2 Corticotropin-Releasing Hormone (CRH-2232 C/G)

Corticotropin-releasing hormone (CRH or CRF) is critical to behavioral and neuroendocrine adaptation to stress. Studies utilizing experimental manipulations of CRH system activity suggest that naturally occurring CRH gene variation may mediate individual variability in behavioral and physiological traits that are key to determining an individual's coping style. One of the most consistent behavioral correlates of CRH system activity is the way an organism approaches novelty and unfamiliar conspecifics (Kalin et al. 2000; Korte et al. 2005). Individuals that readily seek out and investigate novel stimuli are considered "exploratory" or "bold"; those more likely to show fear or withdrawal when confronted with new objects or individuals are described as more "inhibited" or "shy" (Kagan et al. 1988).

CRH haplotype has been shown to predict behavioral inhibition in children (Smoller et al. 2003), and studies in rhesus macaques suggest that human CRH variation may moderate risk for alcohol use disorders, perhaps through the pathway of behavioral inhibition. A rhesus polymorphism (−2232 C/G) that has similar in vitro functional effects to some CRH haplotypes reported in humans (Wagner et al. 2006) predicts decreased CSF levels of CRH, an intermediate phenotype demonstrated in individuals or strains characterized as being particularly extroverted, aggressive, or bold. Infant macaques carrying the G allele are characterized as being more exploratory and bold (Barr et al. 2008b), and, following adolescence, males that are G allele carriers exhibit a more bold and active response to an unfamiliar conspecific. This allele also predicts high-risk alcohol consumption and suggests that CRH variation may influence early or uncontrolled alcohol consumption in human populations (Fig. 1).

4.2 "Relief Drinking": Gene × Stress Interactions

While genetic variation is known to be an etiological factor for alcohol use disorders, not all children of alcohol dependent individuals develop alcohol problems. There is accumulating evidence that genetic and environmental factors interact to determine susceptibility to stress-related disorders later in life (Caspi and Moffitt 2006), a phenomenon is likely to be relevant to the addictions. Rhesus macaques provide opportunity to examine gene × environment (G × E) interactions in a controlled, prospective manner. In the rhesus macaque, mothers invest much of their energy into defending, comforting and caring for their infants, and this maternal buffering appears to be critical to normal infant development (Suomi 1982). In the so-called "peer rearing" condition, subjects are removed from their parents at birth and reared with other age-matched infants, so that they develop in the absence of adult influence (Harlow and Suomi 1974; Chamove et al. 1973). Peer-reared monkeys develop strong bonds with their age mates and use them as a base from which to explore. When compared to their mother-reared counterparts, however, peer-reared subjects

exhibit evidence of insecure attachment, higher levels of anxiety, and lower levels of exploration in novel settings (Suomi 1982). And, as in humans, macaques that have been exposed to early adversity (in the form of peerrearing) show long-lasting differences in brain function and behavior and consume higher levels of alcohol, especially during exposures to stress (Spinelli et al. 2007, 2010a, b; Suomi et al. 1976; Higley et al. 1991).

4.2.1 Serotonin Transporter (rh-HTTLPR)

Of particular interest for the study of G × E interactions is variation within regulatory or coding regions of genes encoding stress-responsive signaling molecules, which may contribute to stress vulnerability or resiliency. The serotonin transporter is a protein critical to regulating serotonin function in the brain since serotonin's action in the synapse is terminated by reuptake. In mice, targeted disruption of the serotonin transporter gene results in increased adrenocorticotrophic hormone (ACTH) and corticosterone responses to immobilization stress as well as increased anxiety during the elevated plus maze and light/dark exploration tasks (Li et al. 1999; Lanfumey et al. 2000; Holmes et al. 2003). Gene expression studies demonstrate that monkeys with high levels of stress reactivity have lower gene expression levels for the serotonin transporter (Bethea et al. 2006).

In humans, there is a common, functional repeat length variant in the regulatory region for the serotonin transporter gene (5-HTT). Variation of this serotonin transporter-linked polymorphic region (HTTLPR) predicts certain personality traits related to anxiety, depression, and aggression, such as neuroticism, harm avoidance, and disagreeableness (Lesch et al. 1997; Mazzanti et al. 1998). There is variation in the serotonin transporter gene regulatory region in many nonhuman primate species (Wendland et al. 2005; Lesch et al. 1997). In rhesus, a 21-bp insertion/deletion polymorphism, rh5-HTTLPR, has been shown to alter transcriptional efficiency (Bennett et al. 2002), resulting in decreased serotonin transporter mRNA levels in brains of l/s macaques (Lopez and Higley 2002), which may be further regulated by epigenetic mechanisms (Kinnally et al. 2010). The HTTLPR polymorphism has been studied extensively in both rhesus and human G × E studies (Fig. 2).

While serotonin release following consumption of alcohol is involved in activation of reward pathways, neuroadaptive diminutions in release during withdrawal can lead to pain, dysphoria, and depression (Koob and LeMoal 2001). Alcohol addiction is associated with dysregulated release, synaptic concentrations, and metabolism of serotonin. Agents that modulate serotonin system functioning can be effective in treating some late-onset, Type 1 alcoholics (Johnson 2010), and the HTTLPR s allele has been associated with certain co-morbid neuropsychiatric disorders in addition to alcohol dependence (Kranzler et al. 2002; Parsian and Cloninger 2001).

Studies in multiple, independent laboratories demonstrate that the rh5-HTTLPR s allele predicts anxiety and responses to stress in rhesus macaques (Bethea et al.

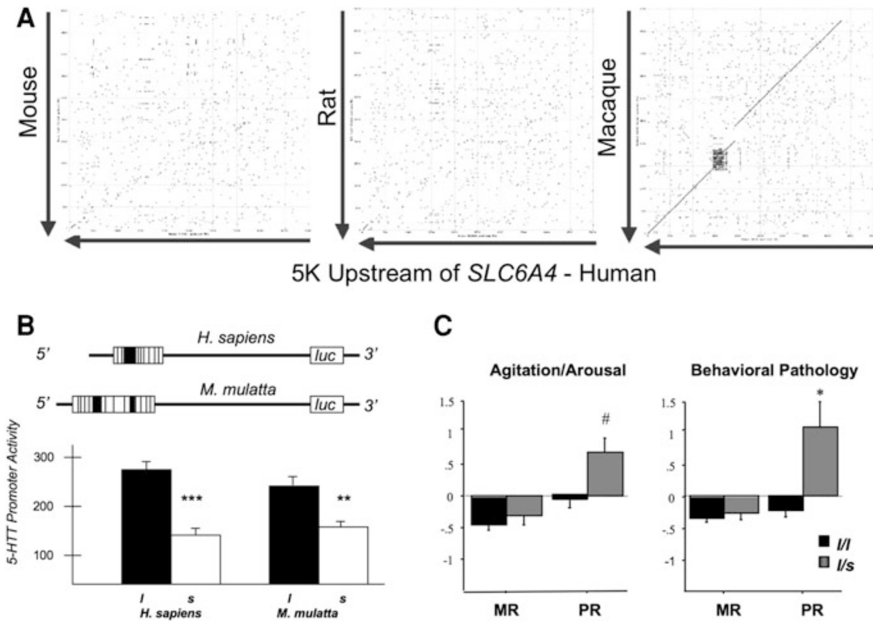

Fig. 2 Utility of the macaque model for performing G × E Interaction studies to model human psychiatric disorders. **a** Alignments of the 5'Flanking region (5 KB) of the human *5-HTT*, or *SLC6A4*, gene with the orthologous regions in mouse, rat and macaque. Each dot indicates a string of eight conserved nucleotides. **b** Like humans, rhesus macaques also have a repeat region that is polymorphic (HTTLPR). In both species, there are long (*l*) and short (*s*) alleles, with the *s* allele conferring decreased transcriptional efficiency relative to the *l* allele. **c** Interactive effects between the HTTLPR *s* allele and early rearing experience (peer-reared, PR, vs. mother-reared, MR) on stress-induced anxiety-like behaviors (Agitation/Arousal and Behavioral Pathology) in infant macaques

2004; Champoux et al. 2002). Further, these studies in rhesus show that HTTLPR genotype can interact with controlled exposures to prenatal stress/alcohol or early life adversity to result in long-lasting differences in stress reactivity, sensation seeking, aggression and alcohol consumption (Barr et al. 2004a, b, c, d; Spinelli et al. 2007; Schwandt et al. 2010; Schneider et al. 2010; Kraemer et al. 2008). Alcohol consumption appears to decrease anxiety in various primate species. Consistent with this, studies have demonstrated that peer-reared female rhesus macaques carrying the s allele exhibit higher levels of alcohol preference than do l/l animals (Barr et al. 2004a), suggesting they may be seeking alcohol for its anxiolytic effects. It may be that independent effects of genotype on anxiety and alcohol response converge to increase alcohol consumption among these subjects (discussed in Sect. 5.1). These studies in rhesus have provided support for the notion that HTTLPR variation interacts with stressful life experiences to moderate risk for stress-related alcohol consumption and other related disorders in humans (Caspi et al. 2003; Caspi and Moffitt 2006).

4.2.2 Neuropeptide Y (NPY-1002 T/G)

The neuropeptide Y (NPY) system is one whose regulation mediates stress adaptation and is, therefore, a candidate system in which functional genetic variation may impact stress resilience (Zhou et al. 2008; Sommer et al. 2010). In response to protracted or repeated periods of stress exposure, NPY is released in key regions of the brain, a mechanism proposed to be important for countering the effects of stress (Heilig and Koob 2007). There is considerable evidence suggesting that NPY regulates alcohol consumption as well (Badia-Elder et al. 2001, 2003). Mice that are deficient for the NPY gene consume more ethanol, while consumption is reduced in transgenic mice overexpressing NPY (Thorsell et al. 2007). In humans, linkage to the chromosomal region containing the NPY gene has been demonstrated (Reich et al. 1998), and there have been associations of NPY variation with both alcohol consumption and dependence (Lappalainen et al. 2002; Mottagui-Tabar et al. 2005). Other studies, however, have failed to replicate this association (Zhu et al. 2003; Zill et al. 2008). This could potentially be indicative of the fact that NPY variation could modify alcohol consumption via interactions with other variables.

Nonhuman primate studies support this. Using the peer rearing primate model of early adversity, a loss-of-function variant in rhesus macaques (NPY-1002 T > G) influences CSF levels of NPY, behavioral arousal during periods of stress, and alcohol consumption (Lindell et al. 2010). In contrast, individuals with no prior stress exposure are unaffected by genotype. The NPY variant also predicts increased alcohol consumption as a function of repeated cycles of alcohol exposure, but only among peer-reared subjects. This suggests a high degree of prior stress exposure to be required for the G allele to produce an effect, raising the possibility that human NPY variation might increase risk for alcohol dependence specifically among individuals with traumatic life experiences or high cumulative levels of stress exposure. In support of this argument, the only study to have reported a link between functional NPY variation and alcohol dependence involved the use of late-onset alcoholics (Mottagui-Tabar et al. 2005) or samples highly represented by war veterans (Lappalainen et al. 2002). Overall, the data from rhesus macaques suggest a role for Neuropeptide Y gene variation in the susceptibility to alcohol-related disorders (Fig. 1) and may further implicate the NPY system as a treatment target in selected individuals.

4.2.3 Corticotropin-Releasing Hormone (CRH-248 C/T)

The CRH system influences not only how individuals approach novel stimuli, but is one that is critical for physiological and behavioral adaptation to stress. However, chronic over-activity of this system can lead to stress-related pathology (Korte et al. 2005; McEwen 2006; Sapolsky 2001; Goldman and Barr 2002). Dysregulation of this system has been linked to a variety of stress-related psychiatric disorders, including depression, PTSD, and alcohol dependence (Gold and

Chrousos 2002; Hundt et al. 2001; Southwick et al. 2005). Studies performed in animal models have shown that an upregulated CRH system can produce anxiety- and/or depression-like phenotypes (Jaferi and Bhatnagar 2007; Kalin et al. 2000; Servatius et al. 2005; Strome et al. 2002). Those performed in rodents show that CRH system upregulation (driven either by genetic variation or environmental factors) leads to escalated alcohol drinking (Hansson et al. 2006; Nie et al. 2004; Sommer et al. 2008). From this body of work, it is inferred that upregulated activity of the CRH system is critical for the transition from impulsive to compulsive alcohol use and, therefore, addiction. Based on these findings, CRHR1 antagonists have been proposed for the treatment of alcohol dependence (Egli 2005; Heilig and Koob 2007).

Most studies demonstrating a role for CRH system upregulation in driving alcohol seeking have been performed in rodents. However, the relative levels of expression and distributions of key mediators of stress responses differ between rodents and catarrhine primates (Sanchez et al. 1999). As such, demonstrating a link between increased CRH system activity and individual differences in alcohol consumption in a primate species provides critical support for the notion that rodent findings may translate to humans. There is a CRH promoter SNP in rhesus macaques (−248 C/T) (Barr et al. 2009), which is located in a region that's under purifying selection and which has been demonstrated empirically to be critical for regulation of CRH expression (King and Nicholson 2007). In vitro studies show that the −248 T allele increases cAMP-stimulated CRH promoter activity, but also disrupts glucocorticoid-mediated repression, indicating that this particular SNP would result in augmented CRH expression in response to stress. When effects of −248 C/T genotype on stress responding are examined, the T allele predicts increased behavioral and endocrine responses to stress among monkeys with a history of early stress exposure. A similar pattern is observed for alcohol consumption. The fact that there is a SNP in the corresponding human region (−201 C > T) (http://genome.ucsc.edu/) suggests that CRH promoter variation could interact with environmental stressors to increase stress responding and alcohol consumption in humans (Fig. 1).

There are other CRH alleles (−2232 C/G) that influence alcohol consumption in rhesus macaques. As stated in 4A (Barr et al. 2008a), there is a functional SNP (−2232 C/G) that is associated with exploratory and bold behavior. The study in which this was reported described two major, alternative CRH haplotypes. The −2232 C/G marker was present on one of the major haplotypes and was shown to diminish sensitivity to low corticosteroid concentrations. This functional effect would be predicted to result in tonic regulation of CRH expression under basal, non-stressed conditions. On the other hand, the −248 C/T SNP discussed here would be predicted to drive increased *phasic* CRH expression, specifically in response to "stress". Whereas −2232 C/G predicts low baseline CSF CRH, high baseline ACTH, bold behavior, and high-risk drinking, the −248 T allele results in enhanced stress reactivity and stress-induced alcohol consumption. In this sense, while the −2232 G allele would be a good candidate for modeling risk for alcohol abuse or early-onset alcoholism (driven by reward drinking), the human functional equivalent to −248 C > T would be predicted to impart risk for

late-onset alcoholism (driven by relief drinking), a subtype that is more common among stress-exposed or anxious individuals. Together, these studies suggest that functional CRH variants could increase alcohol consumption through distinct and varied mechanisms—either by inducing a novelty—seeking/bold temperament or by enhancing stress reactivity (Fig. 1).

5 Gene × Alcohol Interactions and Addiction Vulnerability

5.1 *Pharmacogenetics of Alcohol Response*

In humans, behavioral responses to alcohol are heritable traits that influence vulnerability to alcohol addiction. Measures such as ataxia or subjective high have been studied in relation to alcohol dependence. A low level of response to alcohol and increased subjective high are vulnerability markers for alcohol dependence, as they are heritable traits that are documented in alcohol dependent subjects as well as in people with a family history of alcohol dependence (Schuckit 1994). Candidate gene studies in humans have shown that individuals homozygous for the HTTLPR l allele experience a decreased level of response to alcohol relative to s allele carriers (Hu et al. 2005; Hinckers et al. 2006). Other studies show that carriers of a non-synonymous SNP in the mu opiod receptor gene (OPRM1 A118G) experience increased dopamine release, euphoria (subjective high) and self-reported stimulation following consumption of alcohol (Ramchandani et al. 2010; Ray and Hutchison 2004).

For ethical reasons, results from alcohol challenge experiments in humans are obtained at relatively low blood alcohol concentrations. Moreover, in humans, alcohol expectations and conditioned effects may influence alcohol response. The fact that rhesus macaques have genetic variants that are functional similar to those that influence alcohol response in humans combined with the fact that responses and specific behaviors can be objectively scored in animal models provides an opportunity to perform controlled pharmacogenetic studies.

Unlike human subjects, nonhuman primates can be studied using a controlled dose of alcohol and a subjective scoring system rather than self-report of subjective response. In rhesus macaques, it has been shown that stress-exposed individuals with the HTTLPR l/l genotype exhibit a decreased level of intoxication (LOR, Schuckit 1994) following administration of a binge dose of alcohol (Barr et al. 2003a). As shown here, studies using alcohol response factors as dependent variables (rather than subjective scores) indicate that alcohol-induced Ataxia is significantly lower among l homozygotes, whereas other alcohol response factors (i.e., Disinhibition and Stimulation) are unaltered. This suggests that HTTLPR l/l genotype may reduce alcohol response (LOR) specifically by influencing ataxia and sedation in human subjects. As stated above, OPRM1 A118G predicts increased alcohol-induced euphoria in humans. A rhesus SNP (rhOPRM1 C77G) that is functionally similar to the human OPRM1 SNP that increases alcohol-induced

euphoria (Miller et al. 2004) is associated with increased ethanol-induced stimulation, a commonly used marker of euphorogenic and positively reinforcing actions of alcohol (Barr et al. 2007). This is observed even at binge alcohol doses, at which sedating effects of alcohol typically predominate.

Individuals with certain behavioral traits will be more likely to consume alcohol in certain environmental contexts. This may be particularly true if the impetus for seeking alcohol is especially reinforced as a result of pharmacogenetic factors, which might occur through independent pathways or even as a result of a single allele. As an example of the latter, carriers of the HTTLPR s allele are more anxious and stress-reactive (Lesch et al. 1997; Champoux et al. 2002; Barr et al. 2004a, b, c, d; Spinelli et al. 2007) and, at the same time, they appear to experience more of the sedating effects of alcohol relative to l/l subjects (Hu et al. 2005; Barr et al. 2003b). This might increase the likelihood that they would "self-medicate" their anxiety by consuming alcohol (See Sect. 4.2 above). As another example, OPRM1 variation predicts novelty-seeking and increased sensitivity to social rejection (Barr et al. 2008a; Way et al. 2009). It also increases alcohol-induced euphoria (Barr et al. 2007; Ray and Hutchison 2004). This would not only suggest that novelty-seeking G allele carriers would be more likely to seek alcohol, but that they may do so to a greater extent in social settings or as a function of social rejection or loss of an attachment source (Fig. 1).

Finally, genetic factors that predict alcohol response are likely to be important considerations for individualized treatment of alcohol use. Numerous studies have demonstrated that the opioid receptor antagonist, naltrexone (NTX), is effective in clinical treatment of alcohol dependence (Kreek et al. 2002). Despite one large negative trial, repeated meta-analyses support NTX efficacy on several drinking variables as well as diminished craving. More recent studies indicate that OPRM1 genotype might be deterministic for NTX response (Anton et al. 2009; Oslin et al. 2003). Several labs have demonstrated that OPRM1 genotype predicts naltrexone response in rhesus macaques as well (Vallender et al. 2010; Barr et al. 2010). One study shows that while alcohol preference is markedly suppressed in 77G carriers, increased preference is observed in 77C homozygous individuals during treatment. This pattern parallels a human study that examined family history of alcoholism as a moderator of naltrexone response under laboratory conditions, and found suppression of self-administration in family history positive subjects, but significantly increased self-administration following naltrexone treatment in family history negative participants (Krishnan-Sarin et al. 2007). Considered together, the data point to an intriguing possibility that the modest overall effect size of NTX in treatment of alcohol dependence reflects a heterogeneity in patient responses, and may be considerably improved in appropriately selected patient populations.

5.2 G × E Interactions and Allostasis

The escalation to excessive alcohol intake is dependent upon shifts in the functioning of neurotransmitter and stress hormone pathways (Heilig and Koob 2007). Since these systems mediate stress responding, their over-activity can recruit

negative affective states, and, therefore, put an individual at risk to seek alcohol and rapidly reinstate high levels of consumption, especially in response to stress. Data from rodent studies indicate that this type of adaptation requires that subjects regularly consume sufficient amounts of alcohol to produce BACs in the 100–200 mg% range (Koob and Le Moal 1997). In addition to the fact that alcohol consumption levels and metabolism are important variables in humans, it is likely that there are individual differences in likelihood of transitioning to the addicted state, and that these are related to genetic vulnerability factors influencing stress response or to environmentally altered thresholds.

Both alcohol exposure and withdrawal are potent activators of the HPA axis (Li et al. 2005; Kinoshita et al. 2001). Cortisol can exert pleiotropic effects by altering gene expression in tissues containing high concentrations of corticosteroid receptors (i.e., hypothalamus, amygdala, and hippocampus). It is possible that alcohol-induced neuroadaptive changes in gene expression could be partially attributable to HPA axis activation. Macaques that have early histories of stress in the form of peer rearing exhibit persistent increases in HPA axis activation. Studies in our laboratory have shown that peer-reared female macaques have augmented HPA axis responses to alcohol and that elevated ACTH levels persisted for weeks following discontinuation of the alcohol consumption study (Barr et al. 2004e). It is also demonstrated that early experience and exposure to alcohol interact with one another among females, such that while there is no effect of rearing condition on alcohol consumption during initial exposures to alcohol, that consumption more rapidly escalates among peer-reared subjects, suggesting that stress exposure may be a risk factor for transitioning to casual to compulsive use of alcohol, particularly among female subjects (Barr et al. 2004a).

Cis-occurring variation within regulatory regions for loci encoding corticosteroid-sensitive genes, especially those that encode mediators or receptors within systems altered during the transition to dependence, might be excellent candidates for examining the effects of genetic variation and G $\times$ E interactions as they relate to alcohol drinking (Fig. 3). Whereas glucocorticoid receptor binding to GREs is important to tonic feedback regulation of the HPA axis via diminution in CRH transcription, it induces both CRH and NPY in the limbic system, a mechanism by which behavioral adaptation to stress is thought to occur. Chronic alcohol use results in dysregulation of the HPA axis, and there is evidence from rodent studies that perturbation of the CRH and NPY systems results from repeated cycles of alcohol intake and withdrawal (reviewed in Koob 2003). Further, it has been demonstrated that the dysregulation of these systems underlies transition to the addicted state. Studies in rhesus show that NPY variation that disrupts a functional GRE interacts with repeat cycles of alcohol availability and deprivation, such that stress-exposed carriers of the loss-of-function G allele exhibit an escalation in alcohol intake (Lindell et al. 2010). This is potentially indicative of genotype-mediated inability to recruit the NPY system in response to induction of the CRH system in subjects consuming high levels of alcohol. These data may suggest that these subjects would more easily transition to the addicted state. Whether humans

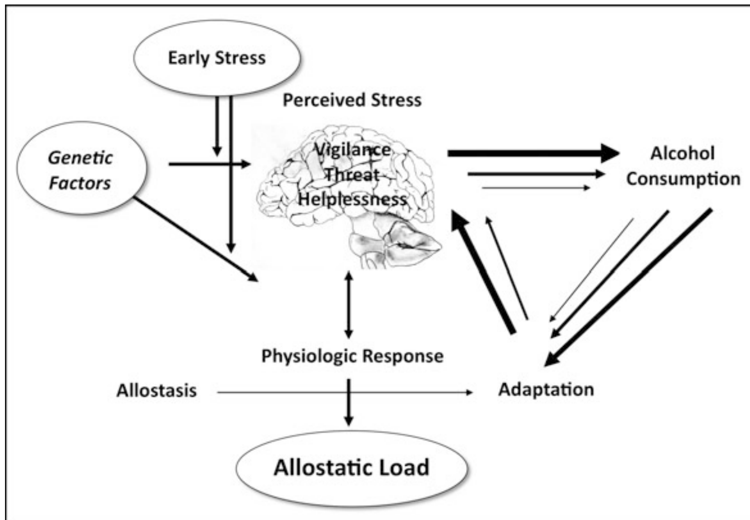

Fig. 3 Proposed model for vulnerability to alcohol-induced allostatic load. Genetic factors may be associated with differences not only in alcohol intake but in activation of physiological systems in response to alcohol challenge. This can be due to differences in alcohol-induced HPA axis output or to increased corticosteroid sensitivity. This may be especially true among subjects who have genetic vulnerability factors that make them more prone to allostatic breakdown of the systems dysregulated in the post-dependent state, for example NPY

with loss-of-function NPY polymorphisms will have similar susceptibilities has not yet been determined.

6 Role of Genetic Selection: Understanding the Origins of Alcohol Use Disorders in Modern Humans

There are a number of research groups that have been investigating genetic variations in the rhesus macaque that contribute to the expression of traits that have been linked with human alcohol problems and other psychiatric disorders (i.e., stress reactivity, behavioral dyscontrol, aggression and reward seeking/sensitivity). What has emerged from this body of work is the fact that, in many cases, the variants that are identified and studied in the macaque are functionally similar to those present in human populations, and some findings suggest there to be convergent evolution and that these variants may have been maintained by selection in both species (Barr et al. 2008a; Vallender et al. 2008). These data reinforce how the macaque model has proven itself useful for learning about how relatively common genetic variants, which are associated with traits that may be adaptive in certain environmental contexts, can also increase vulnerability to stress-related or alcohol problems.

Fig. 4 Rhesus macaques (*Macaca mulatta)* in close social contact (Cayo Santiago). Among those depicted are a mother-infant dyad. Maintenance of social contact is important to macaque survival, so genetic factors that contribute to variance in this trait may be under selection. Similar genetic variants have been shown to contribute to social attachment/sociality in humans, but may in modern society also increase risk for the addictions

While the field of behavioral genetics is growing rapidly, most of its research is concerned with the identification of "disease alleles" or gene variation underlying what is considered pathological behavior. Its methods and findings, however, can be applied to a long-standing goal of evolutionary anthropology, to understand how changes in allele frequency can affect divergences in primate behavior (Fig. 4). Several studies have identified associations between specific alleles and natural features of behavior and life history strategies. For example, the loss-of-function short (s) allele of the serotonin transporter gene promoter length polymorphism (HTTLPR), which increases risk for developing depression in the face of adversity, has a functional equivalent in the rhesus macaque (see above). In macaques, this allele is associated with increased endocrine and behavioral stress reactivity as a function of stress exposure, often in a sexually dichotomous manner (Barr et al. 2004a, b, c, d; Spinelli et al. 2007; Schwandt et al. 2010). Therefore, this variant appears to increase risk for developing psychopathology, particularly in the context of stress. Despite this, these variants have been maintained in both humans and in rhesus (in addition to some other nonhuman primate species). Moreover, in human populations in which the s allele is rare, another loss-of-function variant on the L allele background (LA > LG) is present at a higher frequency (Hu et al. 2006). In humans, there is also a VNTR in the second intron, which appears to be functional (Fiskerstrand et al. 1999). This VNTR is present in a number of primate and non-primate species and is polymorphic in a number of hominoid species (Soeby et al. 2005).

Although SNPs are not necessarily conserved across species, there are instances in which functionally similar SNPs occur in the human and rhesus macaque

(Barr et al. 2008a; Vallender et al. 2008; Miller et al. 2004). It has been demonstrated that gain-of-function 5-HTT SNPs have arisen and been maintained in both rhesus and in humans, suggesting that both gain- and loss-of-function variants may be under selection in primates (Vallender et al. 2008). It is of interest that 5-HTT variation not only predicts individual differences in impulse control and stress reactivity (Barr et al. 2004a, b, c, d; Champoux et al. 2002; Schwandt et al. 2010), but that it is also associated with adaptive traits in free-ranging macaques, such as earlier male dispersal (Trefilov et al. 2000) and male reproductive timing (Krawczak et al. 2005). Whether allelic variation at 5-HTT predicts "adaptive" traits in humans has not been elucidated.

An individual that readily approaches novel objects or conspecifics may do well in certain social situations, but may face higher risk of predation or attack than a more cautious, harm-avoidant individual. Such behaviors might, therefore, be predicted to confer selective advantage at particular developmental or life history stages and in certain environmental contexts. Moreover, because of differences in their behavioral and physiologic responses to stress, the types of stress-related pathology to which bold, proactive individuals and harm-avoidant, reactive individuals are vulnerable are distinct. Whereas the latter are at risk for internalizing disorders, such as depression and anxiety, the former are more likely to develop externalizing conditions, primarily characterized by impaired impulse control (Korte et al. 2005). Such traits are known to impart risk for alcohol use disorders. In humans, anxiety is a risk factor for developing alcohol problems, and stress exposures can lead to craving and relapse (Barr and Goldman 2006; Sinha and Li 2007). It is also known that impulsivity or behavioral dyscontrol can predispose individuals to early and uncontrolled alcohol intake (Barr and Goldman 2006; Goldman et al. 2005).

The CRH locus is one at which variation would be predicted to increase stress adaptation or modify behavior in a manner that is adaptive, but that may also moderate risk for stress-related disorders in modern humans. In macaques, the two most common haplotypes are yin-yang, or alternative haplotypes. The maintenance of these divergent haplotypes over time is suggestive of the fact that they have been subject to selection such that at least one of the alleles on each background is being selected—possibly in a particular environmental context—while the rest are hitchhiking. Several studies in humans (Baerwald et al. 1999; Shimmin et al. 2007) have shown there to be evidence for selection at the CRH locus, in which, similar to the rhesus macaque, alternative, yin-yang haplotype clades are observed (Barr et al. 2008a). As in the rhesus macaque, the major human CRH haplotypes have been shown to vary in terms of their in vitro promoter activity, and among the observed differences are those pertaining to glucocorticoid-sensitivity (Wagner et al. 2006). In rhesus macaques, carriers of a CRH −2232 G allele consume higher levels of alcohol when tested with age-matched peers in a social group, a proposed model for high-risk, impulsivity-related alcohol consumption (Barr et al. 2008a). They also exhibit lower levels of the serotonin metabolite, 5-HIAA, a neurochemical endophenotype observed both in macaques exposed to early life stress and among individuals with early-onset, Type II alcoholism (Higley et al. 1991, 1996a, b). It may be that, in humans, genetic variation that altered CRH system function could

influence multiple behavioral dimensions (i.e., both neuroticism and extraversion) and that variants that placed an individual at the extremes of these spectra (i.e., inhibited and anxious/stress-reactive vs. bold/impulsive and novelty-seeking) could increase the risk for developing alcohol use disorders. Of note, studies that examine effects of CRHR1 haplotype demonstrate both evidence for selection (Nelson et al. 2010) and a moderating effect of haplotype as it relates to stress-induced alcohol drinking (Nelson et al. 2010; Blomeyer et al. 2008).

As another example, in both rhesus and in humans, there are non-synonymous SNPs in the portion of the OPRM1 gene that encodes the N-terminal domain of the receptor (C77G in rhesus macaque and A118G in human), and these SNPs have been observed to confer similar functions in vivo (Barr et al. 2007; Chong et al. 2006; Ray and Hutchison 2004). In humans, the118G allele is suspected to increase the likelihood that an individual will abuse alcohol because it increases alcohol-induced dopamine release and subjective euphoria (Ramchandani et al. 2010; Ray and Hutchison 2004). We have shown that rhesus carrying the 77G allele exhibit increased alcohol-induced stimulation (a marker for the euphorogenic effects of alcohol) and that G allele carriers also consume more alcohol in the laboratory (Barr et al. 2007). It would stand to reason that OPRM1 variation might predict sensitivity to natural rewards as well. Based on the fact that these two variants confer similar functional effects, that both are observed at relatively high frequencies, and, further, that there is an extended region of LD with the A118G allele in humans (Luo et al. 2008; Zhang et al. 2006; Pang et al. 2009) it might be hypothesized that they have evolved as result of similar selective pressures in the two species. Data to directly address this hypothesis are presently not available. However, studies performed in the macaque demonstrate this variant to predict behaviors that could theoretically be under selection. The 77G allele predicts aggressive behavior (Miller et al. 2004), and G allele carriers form stronger attachment bonds with their mothers during infancy (Barr et al. 2008a), especially as a function of repeated maternal separation. It is of interest that the effects reported to occur during repeated exposures to maternal separation and reunion are similar to those that you might observe during periods of alcohol intake and withdrawal. Similar effects of OPRM1 genotype on the expression of social attachment have recently been demonstrated in human children, showing increased quality of parent–child relations as a function of parental unavailability or inconsistency (Copeland et al. 2011). These types of studies highlight how traits that could have conferred selective advantage at some point in the evolutionary history of humans can increase risk for addictive disorders in modern society.

7 Sexually Dichotomous G × E Interactions

From an evolutionary perspective, the roots of psychopathology may lie in the different strategies that have evolved for coping with environmental challenge (Korte et al. 2005). Because selective pressures differ between the two sexes,

males and females exhibit differences in their responses to environmental challenge (Eme 2007; Woody and Eagly 2002). In some ways, the strategies adopted by males and females parallel those described in the Hawk-Dove model: an aggressive/bold strategy (Hawk) opposed by a non-aggressive, cautious strategy (Dove) (Smith and Price 1973). The Hawk–Dove model maps well onto the proposed genetic structure of externalizing and internalizing human psychiatric (Kendler et al. 2003), including Types 1 and 2 alcohol dependence. This, combined with the observation that females are generally more prone to internalizing disorders, such as anxiety and affective disorders, while males are more likely to develop externalizing disorders, such as antisocial behavior and substance use disorders (Cale and Lilienfeld 2002; Williams et al. 1995) underscores the notion of psychopathology as an outcome of the response to stress, and suggests that in addition to genetic and environmental variables, an individual's sex is likely to play an important role.

The serotonin transporter-linked polymorphism is one for which variation results in sexually dichotomous qualitative and quantitative G × E interactions, and rhesus studies were the first to demonstrate these. Even prior to pubertal development, the HTTLPR s allele predicted increased stress response, but only among females with histories of early adversity (Barr et al. 2004a, b, c, d). Responding to social threat is one domain in which males and females are likely to adopt different adaptive solutions, such as those ascribed to the Hawk-Dove model. Recently, it has been shown that male adolescents with the *s* allele who were exposed to early life stress are more likely to respond aggressively towards an unfamiliar conspecific, a risky response that, under certain circumstances, would also be adaptive (Schwandt et al. 2010). Of relevance to alcohol dependence, it may be that the *s* allele confers risk to the two subtypes of alcoholism that are sex-dependent. Alcohol response, which is also moderated by HTTLPR genotype, also likely plays a role.

8 Summary

Because of their complex social structures, behaviors, and genetic similarities to humans, nonhuman primates are useful for studying how genetic factors influence alcohol consumption. The neurobiological systems that influence addiction vulnerability may do so by acting on alcohol response, reward pathways, behavioral dyscontrol, and vulnerability to stress and anxiety. Rhesus macaques show individual differences in alcohol response and temperament, and such differences are influenced by genetic variants that are similar functionally to those present in humans. These polymorphisms also predict alcohol consumption in certain environmental contexts. Candidate gene-based studies performed in nonhuman primates appear to have translational value for investigating effects of genetic variation on traits that increase risk for alcohol use and for understanding how

genetic variation modifies treatment response (Barr et al. 2004a, d, 2009, 2010; Lindell et al. 2010).

This body of work has not only been critically important in arguments for validity of human G $\times$ E studies (Caspi et al. 2010), but it has also provided a solid foundation for supporting studies aimed at identifying novel genetic variants in rhesus macaques that were good candidates for modeling how genetic and environmental variables interact to influence alcohol consumption in modern humans.

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Genetically Selected Alcohol Preferring Rats to Model Human Alcoholism

Roberto Ciccocioppo

Abstract Animal models have been successfully developed to mimic and study alcoholism. These models have the unique feature of allowing the researcher to control for the genetic characteristics of the animal, alcohol exposure and environment. Moreover, these animal models allow pharmacological, neurochemical and behavioral manipulations otherwise impossible. Unquestionably, one of the major contributions to the understanding of the neurobiological basis of alcoholism comes from data that have been obtained from the study of genetically selected alcohol preferring rat lines and from the consequences that alcohol drinking and environmental manipulations, (i.e., protracted alcohol drinking, intoxication, exposure to stress, etc.) have on them. In fact, if on the one hand genetic factors may account for about 50–60% of the risk of developing alcohol dependence, on the other hand protracted alcohol exposure is a necessary precondition to actually develop the disease, while environmental vulnerability factors may be crucial for disease progression. The present article will offer an overview of the different genetically selected alcohol preferring rat lines developed and used to study alcoholism. The predictive, face and construct validity of these animal models and the translational significance of findings achieved through their use will be critically discussed.

Keywords Alcohol preferring rat · Genetic selection · Animal models · Alcohol drinking · Relapse · Self-administration

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R. Ciccocioppo (✉)
Pharmacology Unit, School of Pharmacy, University of Camerino,
Madonna delle Carceri 9, 62032 Camerino, MC, Italy
e-mail: roberto.ciccocioppo@unicam.it

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Abbreviation

UChB	Universidad de Chile "Bebidores" high alcohol drinking line
UChA	Low alcohol drinking line
AA	Alko Alcohol preferring
ANA	Alko, NonAlcohol preferring
P	Alcohol Preferring
NP	Alcohol NonPreferring
sP	Sardinian alcohol Preferring
sNP	Sardinian alcohol NonPreferring
msP	Marchigian sardinian alcohol Preferring
HAD	High Alcohol Drinking
LAD	Low Alcohol Drinking
ADE	Alcohol Deprivation Effect

Contents

1 Introduction 252
2 History 253
3 Genetically Selected Alcohol Preferring Rats: Predictive Validity 255
4 Genetically Selected Alcohol Preferring Rats: Face Validity 257
5 Genetically Selected Alcohol Preferring Rats: Construct Validity 260
6 Conclusions and Remarks 262
References 263

1 Introduction

Alcohol dependence develops gradually, occurs over the course of years, and requires prolonged and repeated exposure of the brain to significant blood-alcohol levels. Both preclinical and clinical research have clearly demonstrated that the presence of genetic traits provides an important contribution to the development of this pathological condition (Cloninger et al. 1981; Sigvardsson et al. 1996), and recent twin studies estimate the contribution of genetic susceptibility factors to 48–58% (Kendler et al. 1997; Prescott and Kendler 1999). However, whether genetically encoded vulnerability is present or not, the process of actually developing dependence is influenced by a number of other factors, such as drug availability, environmental conditions and stress (Ciccocioppo et al. 2001; Katner et al. 1999; Le et al. 1998; Martin-Fardon et al. 2000, 2010; Monti et al. 1999; Rohsenow et al. 2000). The pathological traits of alcoholism are complex and over the years various theoretical frameworks have been proposed to explain it.

A common consensus has been reached, however, on the concept that alcoholism is polygenic in nature, that are different typology of patients and that pharmacotherapies should be optimized according to the patient subgroup treated (Goldman et al. 2005; Heilig and Egli 2006; Heilig et al. 2011). Translated into preclinical research, all these levels of complexities are such that they cannot be mimicked by univocal experimental protocols or laboratory animal models. Nevertheless, while it is recognized that animal models of alcoholism may not be entirely congruent with the human condition, it should be agreed that there are minimal criteria that must be met for an animal model to be considered valid. Therefore, as discussed for other psychiatric disorders (McKinney and Bunney 1969; Newport et al. 2002; Willner 1984) an animal model must resemble the human condition in several respects: (1) should be sensitive to amelioration or attenuation of the symptoms by treatments effective in humans, and conversely insensitive to those treatments that are inactive in attenuating the human disorder (*predictive validity*); (2) should mimic the fundamental behavioral characteristics of human alcoholism and should be characterized by the same symptoms profile (*face validity*); (3) the pathology should be triggered by events thought to be important in eliciting the human disorder and should involve similar neurochemical, neurobiological and psychobiological mechanisms (*construct validity*).

Over the years intense work from several research groups has allowed the development of a number of new preclinical procedures and new animal models to integrate the study of the genetics of alcoholism and the role played by the environment in disease progression. The use of rat lines genetically selected for high ethanol preference or excessive alcohol drinking represented one of the first and most important preclinical approaches to the study of alcohol addiction. Another important contribution to the field has come from the development of well validated experimental paradigms to induce excessive alcohol drinking or chronic alcohol intoxication leading to dependence. In fact, if the genetics is a predisposing factor, exposure to repeated intoxicating alcohol experiences is a necessary condition to facilitate the progression from alcohol use to abuse and dependence.

The present article has the objectives of: (1) providing an update on the different rat lines that have been genetically selected for high alcohol preference; (2) to discuss the contribution of these models in advancing our understanding of the genetics the neurobiology and the physiopathology of alcoholism; (3) to provide a critical discussion on the validity of these animal models and the translational significance of findings achieved through their use.

2 History

Historically, the rat is one of the laboratory animals most frequently and widely used to study the physiopathology and the pharmacology of alcoholism. However this animal, especially under continuous exposure does not drink alcohol spontaneously or if it does it consumes low amounts. About 60 years ago, to overcome

Table 1 List of the different genetically selected alcohol preferring and nonpreferring lines available

Rat line	Predictive	Face	Construct	Notes
UChB/UChA	NA	+	+++	Not enough information to evaluate its predictive value
AA/ANA	++	++	+	Insufficient data to evaluate the construct validity of this rat line
P/NP	++	+++	+++	The only line shown to voluntarily drink alcohol to intoxication
sP/sNP	++	++	++	Alcohol preference was co-segregated with heightened anxiety and depressive-like traits
HAD/LAD	++	++	++	Best genetic criteria for line selection
msP	+++	++	+++	Nonpreferring line is not available. The msP showed the highest predictive validity

(NA) Not available, (+) acceptable, (++) good, (+++) very good

this problem, alcohol researchers initiated, at that time, very ambitious programs to obtain new rat lines genetically selected for high alcohol drinking and preference. This objective was achieved by selective mating of animals with a higher spontaneous preference for alcohol. After repeated mating across generations the genetic traits sub-serving excessive alcohol drinking have been permanently segregated into these rat lines. Simultaneously, control nonpreferring lines were selected for extremely low alcohol preference. As a result of this work we have now available at least 6 different genetically selected alcohol preferring and nonpreferring rat lines around the world (Table 1). The first selective breeding program for rats differing in alcohol consumption began in 1950 at Universidad de Chile (UCh) with the development of high alcohol drinking (UChB) and low alcohol drinking (UChA) lines (Mardones and Segovia-Riquelme 1984; Mardones et al. 1953; Quintanilla et al. 2006). About 10 years later another selection program was started at Alko Research Laboratories in Finland where through bidirectional selection two rat lines, AA and ANA, were selected for high (Alko; Alcohol) and low (Alko, Non-Alcohol) alcohol preference, respectively (Eriksson 1968). Following Mardones' (1953) and Eriksson's (1968) pioneering work two similar programs were initiated in USA (Indianapolis, IN) and in Italy (Cagliari, Sardinia). These programs resulted in the development of the alcohol preferring (P) and nonpreferring (NP) rats and of the sardinian preferring (sP) and sardinian nonpreferring (sNP) rats, respectively (Colombo et al. 2006; Li et al. 1979). A few years later, at the University of Indianapolis the same research group initiated a new breeding program for a bidirectional separation of another line of alcohol preferring and nonpreferring rats. This led to the generation of a replicate line named High Alcohol Drinking (HAD) and Low Alcohol Drinking (LAD), respectively (Murphy et al. 2002). Over the years in a few cases these rat lines have been transferred in laboratories other than those where they were originally developed. In the new laboratories these rat lines were re-derived. For example, in 1988 a few pairs of sP rats were donated by Prof Gessa (University of Cagliari, Italy) to colleagues at the University of Camerino

(Italy). In 1998, after 20 generations of selective breeding, at the Department of Experimental Medicine and Public Health of the University of Camerino, these animals have been re-named msP (for details see Ciccocioppo et al. 1999a). This distinction was made for several reasons: first, when the genetic selection from sP started in Camerino the high alcohol drinking phenotype of the original sP line was only partial. In addition, the two breeding programs were carried out under different husbandry conditions and used slightly different selection criteria. Hence, the genotypic and phenotypic characteristics of sP and msP rats might have been different. About 15 years ago another colony derived from sP rats was established at The Scripps Research Institute (La Jolla, CA). For this line the original name sP was maintained and the first scientific article ever published with animals belonging to this colony appeared about five years ago (Sabino et al. 2006).

In the recent years also the Indiana P line was re-derived to obtain an inbred strain (iP) that is now maintained also at the Howard Florey Institute (University of Melbourne). This inbred strain has retained the high alcohol drinking phenotype of the parental line and has been extensively used for pharmacological and for genetic studies (Carr et al. 2007; Cowen et al. 2005; Hargreaves et al. 2011; Kimpel et al. 2007; Rodd et al. 2007).

The first paper on genetically selected alcohol preferring rats by Mardones et al. appeared in 1953 but since than the number of publications on these rat lines has grown constantly and now we can count a few hundred research papers already published. The work conducted with these animals has provided a unique contribution for the understanding of the genetics, the neurobiology and the physio-pharmacology of alcoholism.

3 Genetically Selected Alcohol Preferring Rats: Predictive Validity

In recent years one of the most exciting developments in the field of alcoholism is the introduction of effective medications such as naltrexone and acamprosate (Sass et al. 1996; Volpicelli et al. 1992). These agents proved the feasibility of pharmacological treatment of alcoholism. More recently, other drugs have been tested in the clinic for their ability to reduce ethanol drinking and relapse. The results of these initial studies showed, for example, that ondansetron, an antagonist of the serotonin $5HT_3$-receptor, exerts marked beneficial effects, but did so exclusively in early-onset patients (Johnson et al. 2000a, b). Other drugs of interests are those compounds that modulate central GABAergic transmission or reduce neuronal excitability such as topiramate, gabapentin and pregabalin, as well as the GABA B receptor agonist baclofen (Addolorato et al. 2000; Johnson 2005; Martinotti et al. 2008; Mason et al. 2009; Stopponi et al. 2012). A large body of evidence indicates that genetically selected alcohol preferring rats are highly sensitive to inhibition of alcohol consumption by treatments that have shown efficacy also in humans which may support the predictive validity of these animal models. For example,

naltrexone, a drug extensively used in the clinical practice and that reduces alcohol consumption and cue reactivity in humans lowers alcohol intake also in several lines of alcohol preferring rats. The efficacy of naltrexone was documented in the P (Dhaher et al. 2012), the HAD (Krishnan-Sarin et al. 1998), the sP (Sabino et al. 2006), the AA (Koistinen et al. 2001) and the msP lines (Ciccocioppo et al. 2007; Perfumi et al. 2005). Another drug of proven efficacy in alcoholics is acamprosate (Mann et al. 2008). Studies in iP and in AA alcohol preferring rats confirmed the efficacy of this drug on alcohol drinking thus offering additional evidence for the predictive validity of these animal models (Cowen et al. 2005).

Another example supporting the predictive validity of alcohol preferring rats is 5-HT3 antagonism. Relatively recent studies, in fact, revealed promising therapeutic effects with the 5-HT3 selective antagonist ondansetron that appeared to be particularly effective in early-onset alcoholics (Johnson et al. 2000a, b). Notably, ondansetron was able to reduce alcohol intake also in the P rat. While MDL72222, another selective 5-HT3 receptor antagonist resulted effective in P and in sP rats (Fadda et al. 1991; McKinzie et al. 2000; Rodd-Henricks et al. 2000).

According to the definition of predictive validity, if a medication is inactive in humans alcoholics it should also be inactive in alcohol drinking animals. An interesting example is offered by the selective serotonin 5-HT$_2$ receptor antagonist ritanserin that was shown to be unable to reduce ethanol drinking in patients (Johnson et al. 1996) and also in msP (at that time named sP) rats (Ciccocioppo et al. 1995). In this case, the predictive value of msP rats appeared to be higher than that of AA and P rats in which blockade of 5-HT$_2$ receptors reduced ethanol drinking (Overstreet et al. 1997; Roberts et al. 1998). Of interest is the unusual case of the Selective Serotonin Reuptake Inhibitors (SSRI). In preclinical research, these drugs showed efficacy in almost all experimental animal models used to investigate their effect on alcohol drinking, including genetically selected alcohol preferring rats (Ciccocioppo et al. 1997; Maurel et al. 1999; Murphy et al. 1985; Rezvani et al. 2000). In addition, reinstatement studies, (i.e., resumption of extinguished drug-paired lever responding following extinction or after an imposed period of abstinence) demonstrated that fluoxetine reduces also stress-induced relapse in rodents (Le et al. 1998). Contrary to what animal research predicted, treatment with this class of compounds showed very little, if any, efficacy in humans (Garbutt et al. 1999; Nunes and Levin 2004). If we consider that SSRIs markedly inhibit ingestive behavior in general, one could explain this false positive by hypothesizing that the reduction of ethanol drinking in laboratory animals is an epiphenomenon associated to the anorectic effects of these agents. This could be particularly true for genetically selected alcohol preferring rats because due to their high ethanol consumption (6–8 g/kg day) they retain a considerable amount of calories from alcohol. Hence, their drinking behavior could be highly sensitive to pharmacological manipulation of feeding related mechanisms. Some clinical evidence suggest, however, that SSRIs may have some beneficial effects on ethanol drinking and on other ethanol-related behaviors in patients with a diagnosis of comorbid depression (Nunes and Levin 2004). In a forced swimming test study it was shown that msP and sP rats have a particularly high level of

depressive-like behaviors. Those were reversed by repeated intragastic ethanol administrations or by treatment with the anti-depressant drug desipramine (Ciccocioppo et al. 1999a). These data may suggest that in these rat lines ethanol has an antidepressant-like action that may contribute to their high motivation to drink ethanol for self-medication purposes. This may provide an alternative explanation for which treatment with fluoxetine (Ciccocioppo et al. 1997) or desipramine, removing the depressive-like negative state typical of these animals, may significantly lower their spontaneous ethanol drinking.

4 Genetically Selected Alcohol Preferring Rats: Face Validity

In the Diagnostic and Statistical Manual of Mental Disorders (DSM IV), alcohol dependence is defined as a maladaptive pattern of drug use leading to clinically relevant impairment and distress associated with specific phenomena such as drug intoxication, development of tolerance, occurrence of withdrawal, uncontrollable drug seeking and continuous use of the drug despite knowledge of its negative effects. To have face validity, an animal model of alcoholism has to mimic the fundamental behavioral characteristics of human alcoholism and should be characterized by the same symptoms profile. Some of these characteristics are intrinsically expressed in all genetically selected alcohol preferring rat lines. For example, all of them are characterized by consumption of pharmacologically relevant daily doses (6–8 g/kg even 10 g/kg) of ethanol. In addition, several reports showed that alcohol consumption in these animals is largely concentrated during the active phase (night) of the light dark cycle when intake is organized in bouts of several milliliters of ethanol (up to 10–15 ml of 10% ethanol) leading to blood-alcohol levels (BAL) above 50 mg/dl, thus indicating that intake is largely driven by the pharmacological properties of alcohol (Ciccocioppo et al. 2006). Few studies also evaluated the taste reactive response of these rat lines to alcohol. Results showed, for instance, that compared to ANA controls, naive AA rats make significantly higher levels of ingestive responses to ethanol. A further increase is observed in ethanol experienced AA rats. These two rat lines emit identical aversive responses to alcohol but following a period of acclimation they are reduced in the AA but not in the ANA line (Badia-Elder and Kiefer 1999). A similar finding was reported in a taste reactivity study in msP rats in which it was shown that these animals do not show aversive reactions to ethanol following its passive infusion into the mouth (Polidori et al. 1998). This can, at least in part, explain why msP rats voluntarily drink large amount of alcohol from the very first presentation (Ciccocioppo et al. 2006). Lastly, studies in UChB and in P rats, showed that when compared to their nonpreferring counterparts they have less sensitivity to the aversive effects of alcohol (Froehlich et al. 1988; Quintanilla et al. 2001). Notably, UChB rats appear to be insensitive to the aversive effects of acetaldehyde, the main alcohol metabolites, that in UChA rats produces, instead, a marked conditioned taste aversion (Quintanilla et al. 2002).

Overall these data demonstrate that genetically selected alcohol preferring rats seek ethanol and shape their behavior to obtain pharmacological effects from its intake. This concept is further supported by self-administration experiments showing, for example, that P rats can lever press to infuse alcohol directly into the stomach or the ventral tegmental area of the brain (Gatto et al. 1994; Waller et al. 1984). Consistent findings were obtained also in msP rats in which place conditioning studies revealed that in this rat line intragastric administration of 0.7–1.5 g/kg of alcohol elicited a marked conditioned place preference (Ciccocioppo et al. 1999b). Conversely, in nonselected Wistar rats, intragastric alcohol administration leads to conditioned aversive responses (Fidler et al. 2004).

Another key feature in alcohol addiction is that subjects voluntarily drink intoxicating doses of alcohol that, after abrupt discontinuation of intake, terminates into a withdrawal syndrome. Drinking to intoxication is very difficult to observe in laboratory animals. However, it was shown that P rats can develop physical dependence upon protracted exposure to 10% alcohol under free choice (water vs alcohol) condition (Kampov-Polevoy et al. 2000). Such evidence was not confirmed in msP rats, neither to our knowledge, in other preferring lines. However, this should not be surprising, considering that in msP rats the BALs reached following voluntary ethanol intake generally remain below 100 mg/dl (Ciccocioppo et al. 2006). In fact, as reported in many studies, physical symptoms of alcohol withdrawal appears following intoxication paradigms aimed at reaching BALs of at least 150 mg/dl (Braconi et al. 2009; Hermann et al. 2011; Majchrowicz 1975; Penland et al. 2001; Rimondini et al. 2002). In humans, alcohol withdrawal is also characterized by a number of psychological symptoms that includes agitation, anxiety, depression and dysphoria. Some of these symptoms, (i.e., anxiety- and depressive-like signs) can be detected also in laboratory animals, in which they appear after intoxicating doses of alcohol leading to lower BALs compared to those needed to observe physical withdrawal. In one study examining the behavior of msP rats in the forced swimming test it was shown that naive animals exhibit a longer period of immobility compared to alcohol experienced msP rats allowed to voluntary drink ethanol for 10 days before the forced swimming test. After 10 days of voluntary 10% ethanol drinking, if alcohol is removed from the home-cage for 10 days, immobility score increases again to values similar to those of naive rats. Voluntary ethanol consumption or intragastric administration of appropriate doses of alcohol (6.3 g/kg of ethanol given in 9 boluses of 0.7 g/kg of ethanol) administered during the 24 h preceding the swimming test reduced the immobility time (Ciccocioppo et al. 1999a). Overall these data show that while ethanol exerts an antidepressant-like action at doses that alcohol preferring rats voluntarily take, an imposed abstinence in alcohol experienced animals exacerbate depressive-like symptoms (as expected in human abstinent alcoholics). Of note, in the P rat withdrawal from alcohol is followed by increase in anxiety-like behaviors, another affective sign of abstinence (Kampov-Polevoy et al. 2000). Consistently sP rats, compared to their nonpreferring sNP counterpart, showed higher anxiety-like behaviors that was, however, attenuated following alcohol consumption (Colombo et al. 1995). A similar phenotype was described also

in the msP line in which heightened anxiety was linked to a genetic mutation occurring at corticotrophin releasing hormone receptor-1 gene (Hansson et al. 2006).

Another interesting phenomenon occurring in alcohol preferring rats and that is associated with alcohol abstinence is the occurrence of a alcohol deprivation effect (ADE). If ethanol experienced alcohol preferring rats are withdrawn from ethanol for a few days or weeks, when alcohol access is returned they show a clear shift toward a higher level of drinking. This phenomenon was observed in P, and though to a lower extent in sP and msP rats (Agabio et al. 2000; Perfumi et al. 2005; Rodd et al. 2003). Contrasting findings were described in HAD rats while no ADE was reported in the AA line (Rodd et al. 2009; Vengeliene et al. 2003). The ADE in alcohol preferring rats should be interpreted as the intense motivation of these animals to resume ethanol use following an abstinence period. During disease progression alcohol deprivation experiences are recurrent also in alcoholics. Like in animals, following abstinence episodes, these individuals often report an increasing urge to drink that normally terminates with an uncontrollable severe alcohol intoxication episode. With respect to these characteristics the P and the sP lines appear to more closely mimic human behavior compared to AA and HAD rats.

Clinical studies reveal that conditioning factors and stress may play a major role in maintaining addictive behaviors and in facilitating relapse (Koob and Le Moal 1997; O'Brien et al. 1998). Conditioning hypotheses are based on observations that relapse is often associated with exposure to ethanol-related environmental stimuli. According to this view, environmental stimuli that have become associated with the subjective actions of ethanol by means of classical conditioning throughout an individual's history of ethanol abuse elicits subjective states that can trigger resumption of drug use. Stress may, instead, result in mood dysregulation, disruption of neuroendocrine homeostasis and somatic symptoms, such as insomnia and agitation that may motivate alcoholic patients to resume drinking to alleviate negative affective states. Alcohol preferring rats represent an excellent model to reproduce these complex behavioral traits described in the human literature. For example, it has been shown that msP rats trained to operantly self-administer 10% ethanol or water in 30 min daily session in the presence of discriminative stimuli associated with the availability of ethanol versus water, following an extinction period resume their lever pressing for ethanol, but not for water associated cues. Similar behavior was observed also in nonselected Wistars; however, remarkable line differences in the magnitude and persistence of the response-reinstating effect of ethanol-associated stimuli was observed between the two rat lines (Ciccocioppo et al. 2006). Using slightly different conditioning/reinstatement models identical results have been described also in sP and P rat lines (Ciccocioppo et al. 2001, 2006; Maccioni et al. 2007). Another important finding was that compared to nonpreferring controls or heterogenous Wistars in the preferring lines cue exposure resulted in a more persistent ability to trigger relapse to alcohol seeking also after a protracted period of abstinence (Ciccocioppo et al. 2001, 2006).

These findings not only confirm that the reinforcing properties of ethanol are increased in rats with a genetic predisposition toward heightened ethanol intake but provide evidence that genetically determined alcohol preference extends to

greater responsiveness to the motivating effects of ethanol-associated stimuli. In a recent self-administration study it was also shown that in an extinction-reinstatement paradigm exposure to intermittent foot-shock stress reinstates lever pressing for ethanol in both msP and Wistar rats. However, msP rats showed the highest reinstatement levels following administration of 0.3 mA foot-shock current intensity whereas the maximal responses in Wistars were observed after exposure to 1.0 mA electric current. At 1.0 mA the locomotor behavior of msP rats was impaired because freezing behavior occurred (Hansson et al. 2006). Altogether these data suggest that msP rats are characterized by a heightened sensitivity to stress which may contribute to shape their high ethanol drinking phenotype (Hansson et al. 2006). Of note, in a study where Wistars, AA, HAD and P rats were tested for the alcohol deprivation effect (also a model of relapse) following exposure to foot-shock stress it was found that shock increased alcohol consumption in all rat lines, but the most pronounced effects were observed in the HAD and in the P lines (Vengeliene et al. 2003). Overall these data suggest that genetically selected preferring lines and heterogeneous nonselected rats both show relapse-like behaviors after exposure to stressful stimuli but in the preferring lines the sensitivity appears to be higher.This reflects the results of several clinical studies showing that a large population of alcoholics have lower ability to engage into stress-coping strategies and that resumption of alcohol abuse is often a mechanism to ameliorate the negative affective state in which they precipitate following exposure to anxiogenic stimuli or stress, especially during protracted withdrawal (Bartlett and Heilig 2011; Sinha 2011).

5 Genetically Selected Alcohol Preferring Rats: Construct Validity

An animal model of alcoholism should rely on similar neurochemical, neurobiological and physiological mechanisms and should be sensitive to the same events thought to be important in eliciting the human disorder in order to have construct validity. Many years of clinical and experimental research have demonstrated that alcoholism is a multifactorial disorder where genetic predisposition associated to environmental factors can contribute to the final level of abuse vulnerability. The fact that genetic selection produced animal lines expressing high ethanol drinking phenotype is *per se* an important element of because it shows that, like in humans, vulnerability to abuse ethanol can be inherited. An additional level of validity comes from co-segregation of the excessive drinking phenotype with high anxiety- and depressive-like affective traits as observed in P, sP and msP lines, reflecting pathological conditions described in large alcohol addict subpopulations (Ciccocioppo et al. 1999a; Colombo et al. 1995; Stewart et al. 1993). On the other hand, in the HAD and in the AA alcohol preference has been associated with impulsive traits suggesting that these rat lines may resemble a different population of alcoholic patients like those characterized by early disease onset, high impulsive

behavior and antisocial personality (Enoch 2003; Moller et al. 1997; Sommer et al. 2006; Wilhelm and Mitchell 2008).

An ideal genetic animal model of alcoholism should carry the same genetic traits that are linked to alcoholism in humans. In recent years a wealth of work has been carried out to understand the genetic basis of alcoholism and a lot of information has been collected. It is now clear that alcoholism is a multigenic disorder and various genetic polymorphisms have been associated to alcohol abuse vulnerability. The most compelling evidence is that polymorphism in the genes encoding different alcohol and acetaldehyde dehydrogenase isoforms can dramatically affect an individual's risk to develop alcoholism. For instance, the slow isoform acetaldehyde dehydrogenase2 (ALDH2) and the fast isoform alcohol dehydrogenase (ADH) are protective against alcoholism (Higuchi 1994; Thomasson et al. 1991; Tu and Israel 1995). Consistent with this finding in humans it was shown that the low alcohol drinking line UChA carries a slow form of ALDH that is not present in the alcohol preferring UChB, thus allowing these latter to consume higher doses of alcohol without experiencing aversive reactions (Quintanilla et al. 2005a, b). Other genes linked to increased vulnerability to develop alcoholism are those encoding for specific variants of $GABA_A$ receptor (*GABRA2* and *GABRG3*), muscarinic cholinergic (*CHRM2*) receptors, opoid receptors (OPRK1, PDYN, OPRL1), alpha-synuclein protein (SNCA), neuropeptide Y (NPY) etc. (Dick et al. 2004, 2006, 2008 ; Foroud et al. 2007; Lappalainen et al. 2002; Xuei et al. 2006, 2007, 2008). Polymorphisms at dopamine D2, μ-opioid receptor, and serotonin transporter genes have also been associated with increased vulnerability to develop alcoholism and with a different response to pharmacological interventions, see for review (Heilig et al. 2011). The significance of these genes in alcohol preferring rats have not been systematically investigated yet. However, in qantitative trait loci (QTL) mapping studies it was found that in inbred P (iP) rats chromosome 4 is associated with alcohol preference. Compared to the nonpreferring inbred counterpart (iNP) approximately 11% of the phenotypic variability appears to be linked to this QTL. Noteworthy, several candidate genes identified in the human studies (i.e., SNCA, NPY, CHRM2, TAS2R16 and ACN9) have homologs located on this rat chromosome (Carr et al. 1998, 2007; Liang et al. 2010).

In a relatively recent study an extensive gene mapping study has been undertaken in msP rats. The most striking evidence obtained in these animals is that they carry with high correlation two single-nucleotide polymorphisms on the promoter region of the gene encoding for the CRF_1 receptor. Combining this finding with the observation that msP rats have a higher expression of CRF_1 receptor mRNA and CRF_1 receptor protein density in various brain regions one may speculate that the gene variant identified in msP rats may be functionally relevant (Hansson et al. 2007, 2006). Interestingly, in a recent investigation it has been reported that also in humans, polymorphisms at the level of the promoter region for the CRF_1 receptor gene are linked to alcohol use disorder. For example, in an adolescent at risk population it was found a significant correlation between two SNPs (Reference SNP IDs-number; rs242938 and rs1876831), binge drinking and lifetime prevalence of drunkeness (Treutlein et al. 2006). The same association was found in an

independent sample of adult alcohol dependent patients in which rs1876831 polymorphism was linked to higher levels of alcohol drinking (Blomeyer et al. 2008; Chen et al. 2010; Treutlein et al. 2006).

Altogether these findings suggest that alcohol preferring rats and humans, at least in part, share common genetic predisposing factors to alcoholism.

6 Conclusions and Remarks

Genetically selected alcohol preferring rat lines were developed several decades ago, when other genetic tools such as engineered mice and sophisticated high-throughput gene expression and gene sequences analysis for human studies were not available yet. For several years these rat lines have offered a unique opportunity to investigate the genetics of alcoholism. Often observations in genetically selected alcohol preferring rats have inspired hypothesis driven genetic studies in humans or have stimulated further *ad hoc* genetic studies in engineered animals. These rat lines have also offered a unique help for advancing our knowledge of the neurobiology of alcoholism and has allowed the possibility to carry out pharmacological studies to evaluate drug effects on alcohol drinking. In fact, non selected rodents and laboratory animals in general do not readily drink alcohol and in most of the cases their consumption is too low to pharmacologically manipulate alcohol drinking or to evaluate the neurobiological consequences of alcohol exposure. These limitations have been now, partially overcome with the more recent development of nongenetic animal models of excessive drinking (see chapter by Becker).

A wealth of data collected over the years of research suggests that the alcohol preferring rat may indeed represent an animal model of alcohol abuse endowed with good predictive, face and construct validity. Nevertheless, the clinical translational value of results collected in alcohol preferring rats remains less clear. In fact, on the one hand some genetic traits linked to alcohol abuse vulnerability seems to be shared by humans and alcohol preferring rats. On the other hand, the breeding and selection programs of these rat lines were merely based on alcohol preference criteria. It is unlikely, therefore, that human alcoholism which is characterized by complex phenotypic traits such as drinking to intoxication despite the negative consequences associated to it and rodent alcohol preference are subserved by the exact same genetics. In this regard, among the different alcohol preferring rat lines only the P seems, under certain experimental condition, to spontaneously drink enough alcohol to achieve intoxication and dependence (Kampov-Polevoy et al. 2000).

Another consideration is that alcoholism is a heterogeneous disorder to which several genetic factors may contribute. Indeed, several phenotyping and genotyping criteria have been proposed to group alcoholic patients into more homogeneous subpopulations (Cloninger 1987; Heilig et al. 2011). Similarly, also alcohol preferring rat lines appear to differ in their phenotypes. For example the P, the sP and the msP lines are characterized by high anxiety-like traits; the AA and the LAD do not. sP and msP rats appear also to show depressive-like

symptoms that are medicated by alcohol intake (Ciccocioppo et al. 1999a). While in the UChB/UChA lines the pharmacokinetics of alcohol seems to play a critical role (Quintanilla et al. 2006). Hence, it is likely that the different lines of genetically selected alcohol preferring rats may resemble subpopulation of alcoholics; generalization of the findings in these animals may be of questionable value.

At present only two drugs have been FDA approved for the treatment of alcoholism and relapse prevention; namely naltrexone and acamprosate. These two agents have been extensively tested in animal models of alcohol drinking including genetically selected rats. In most of these experiments results correctly predicted drug efficacy in humans (Cowen et al. 2005; Dhaher et al. 2012; Koistinen et al. 2001; Krishnan-Sarin et al. 1998; Perfumi et al. 2005; Sabino et al. 2006). These findings support the translational value of pharmacological findings in alcohol preferring rats. However, as described in previous paragraphs, there are other examples (i.e., the case of 5-HT2 receptor antagonists or the case of SSRIs) in which preclinical data in genetically selected alcohol preferring rat lines was unable to clearly predict actual clinical outcomes (Ciccocioppo et al. 1995, 1997; Johnson et al. 1996; Maurel et al. 1999; Murphy et al. 1985; Overstreet et al. 1997; Rezvani et al. 2000; Roberts et al. 1998).

In conclusion, there should be no doubt that genetically selected alcohol preferring rat lines represent a very useful model to study alcoholism. Human alcoholics, on the other hand, consist of a heterogeneous population of individuals with alcohol abuse as a common problem. These individuals are characterized by genetic variability, life history, drug exposure (i.e., time of exposure and amounts), environment, etc. All these factors are important and they all contribute to shaping the trajectory of disease progression. Considering these levels of complexities it appears unlikely that a single animal model of alcoholism or a single line of alcohol preferring rats may mimic the human condition in a satisfactory way. Rather, it is reasonable to believe that any different alcohol preferring rat line, or animal model, may catch some aspects of the human disorder but not all. To maximize the translational power of preclinical research it is important, therefore, to collect evidence from as many different animal models as possible. The different lines of alcohol preferring rats may be viewed as important tools to achieve this objective.

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Advanced Transgenic Approaches to Understand Alcohol-Related Phenotypes in Animals

Ainhoa Bilbao

Abstract During the past two decades, the use of genetically manipulated animal models in alcohol research has greatly improved the understanding of the mechanisms underlying alcohol addiction. In this chapter, we present an overview of the progress made in this field by summarizing findings obtained from studies of mice harboring global and conditional mutations in genes that influence alcohol-related phenotypes. The first part reviews behavioral paradigms for modeling the different phases of the alcohol addiction cycle and other alocohol-induced behavioral phenotypes in mice. The second part reviews the current data available using genetic models targeting the main neurotransmitter and neuropeptide systems involved in the reinforcement and stress pathways, focusing on the phenotypes modeling the alcohol addiction cycle. Finally, the third part will discuss the current findings and future directions, and proposes advanced transgenic mouse models for their potential use in alcohol research.

Keywords Genetics · Conditional knock-outs · Behavior · Alcohol addiction cycle · Initiation · Maintenance · Craving · Relapse · Brain neurotransmitter systems

Abbreviations

2AG	2-arachidonoyl glycerol
5HT	Serotonin
5HTT	Serotonin transporter
ADE	Alcohol deprivation effect
ACTH	Adrenocorticotropic hormone

A. Bilbao (✉)
Faculty of Medicine Mannheim, Institute of Psychopharmacology,
Central Institute of Mental Health, University of Heidelberg,
ZI J5, 68159 Mannheim, Germany
e-mail: ainhoa.bilbao@zi-mannheim.de

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AEA	Anandamide
CNS	Central nervous system
CORT	Cosrticosterone
CRF	Corticotropin releasing factor
CTA	Conditioned taste aversion
CPP	Conditioned place preference
DAGL	Diacylglycerol lipase
DAT	Dopamine transporter
DID	Drinking in the dark
ECS	Endocannabinoid system
FAAH	Fatty acid amide hidrolase
GP	Globus pallidus
HIC	Handling-induced convulsions
HPA	Hypothalamic-pituitary adrenal axis
KD	Knock-down
KI	Knock-in
KO	Knock-out
LORR	Loss of righting reflex
MAGL	Monoacyl glycerol lipase
Sert	Serotonin
THC	Delta (9)Tetrahydrocannabinol
Transg +	Transgene overexpression
VTA	Ventral Tegmental Area

Contents

1 Introduction 273
2 Contribution of Genetically Manipulated Mice Models to Alcohol-Related Phenotypes: Focus on Neurotransmitter Systems 274
2.1 Glutamate System 275
2.2 GABA System 278
2.3 Dopamine and Serotonin Systems 281
2.4 Endocannabinoid System 285
2.5 Opioid System 287
2.6 Corticotropin Releasing Factor System 290
3 Future Directions: Advanced Genetic Models to Study Alcohol-Related Phenotypes 292
3.1 Glutamate System 293
3.2 GABA System 296
3.3 Dopamine and Serotonin Systems 296
3.4 Endocannabinoid System 296
3.5 Opioid System 297
3.6 Corticotropin Releasing Factor System 297
4 Conclusions 297
References 298

1 Introduction

Alcohol addiction is a complex disorder affecting several neurotransmitter systems in the brain's reinforcement and stress pathways (for review Vengeliene et al. 2008; Spanagel 2009). In addition to the pharmacological and molecular studies, the contribution from transgenic and knock-out mouse models developed during the past 20 years has been extremely important for a better understanding of the neurobiology of alcohol addiction. This is also reflected by a pronounced increasing publication trend for papers with genetically modified animals in the addiction field (Helinski and Spanagel 2011). These studies have provided crucial information for the understanding of the neurobiological pathways mediating the effects of alcohol on the central nervous system (CNS). On the other hand, considering the complexity of the aetiology of alcohol addiction which involves multiple environmental and genetic interactions, the need for adequate behavioral paradigms to investigate the role of specific genes is of great importance. In this regard, a parallel progress done in the behavioral field during the previous years, aiming to isolate and analyze the complexity of alcohol addiction, has resulted in the development of animal models highly sophisticated mimicking each particular phase of the alcohol addiction cycle (Sanchis-Segura and Spanagel 2006; see also the chapter by Martin-Fardon and Weiss). Thus, the initiation and maintenance of alcohol consumption, and alcohol-seeking during abstinence and relapse-like drinking can be successfully mimicked or modeled in the laboratory (Table 1). Consilience of rodent and human phenotypes relevant for alcohol addiction as outlined in Table 1 has been recently reviewed in great detail (Crabbe et al. 2010a; for alcohol sensitivity Crabbe et al. 2010b; for alcohol consumption and reward Leeman et al. 2010 and Stephens et al. 2010; for alcohol-seeking and relapse Vengeliene et al. 2009; for acute and protracted withdrawal Heilig et al. 2010).

Given the current state of advanced genetic technologies and behavioral tools by which to evaluate phenotypes relevant for alcohol addiction, we are now in the position to combine both approaches to improve the understanding of the neurobiological basis of the alcohol addiction disorder. The aim of this chapter is to provide the reader an overview of the current situation and the progress made in this field. To this end, a comprehensive summary of the current findings obtained from studies of mice harboring mutations in genes involved in the aforementioned alcohol-related phenotypes will be presented. The target genes selected here will include the main neurotransmitter and neuropeptide systems involved in the reinforcement and stress pathways, and the alcohol-related phenotypes listed in Table 1. For another very comprehensive review on alcohol-related genes and contributions from studies with genetically engineered mice we refer to Crabbe et al. (2006) who reviewed 141 published reports of effects of 93 genes on responses to alcohol. Finally, we will propose the use of more advanced genetic mice models that could potentially improve our understanding of the neurobiological mechanisms underlying alcohol addiction.

Table 1 Animal models for studying the different phases and traits of the alcohol addiction cycle

PHASE		TEST	DESCRIPTION
Initiation	Sensitivity	Loss of Righting Reflex (LORR)	Measures CNS sensitivity in response to the high /hypnotic effects of alcohol, usually estimated as the duration of sleep time.
	Reinforcement	Conditioned place preference (CPP)	Measures the positive motivational properties of alcohol. After a few pairings of conditioning, a CPP response to the alcohol-associated compartment can be measured in a drug-free state.
		Acquisition of voluntary alcohol consumption	Initial training of taste adaptation until pharmacologically relevant amounts of alcohol are orally self-administered in the homecage. The measurements include the amount consumed (expressed as grams per kilogram), preference of alcohol over water and the total fluid intake.
		Acquisition of operant self administration	Initial training to learn an operant response for alcohol in an operant (Skinner) box by the so-called saccharin or sucrose fading procedure, where ethanol concentrations are increased, being presented first with saccharin, and afterwards without saccharin. Different schedules of reinforcement can be used.
Maintenance	Chronic Intake	Long-term consumption	After the acquisition phase, alcohol consumption or lever pressing becomes more stable, with a tendency towards a decline, which is more obvious during voluntary (and not operant) intake.
		Stress or dependence induction effects	Factors that, by affecting reinforcement processes, can lead to excessive alcohol motivation and consumption.
Craving & Relapse	Seeking	Cue, priming, stress-induced reinstatement of alcohol seeking	A procedure for studying alcohol-seeking behaviour. Alcohol-experienced animals are subjected to extinction. 3 events can then reinstate responding: (i) drug priming (or the injection of a small but pharmacologically effective dose of ethanol), (ii) stress, and (iii) conditioned stimuli.
	Relapse	Alcohol Deprivation Effect (ADE)	A model for the study of relapse-like behaviour. Consists on a transient increase in alcohol intake after a period of forced abstinence in alcohol-experienced animals. The ADE can be observed in numerous species including rats, mice, and monkeys.
	Withdrawal	CNS hyperexcitability	Symptoms produced after abrupt cessation of long-term alcohol consumption. The withdrawal severity is commonly scored by means of handling-induced convulsions.

2 Contribution of Genetically Manipulated Mice Models to Alcohol-Related Phenotypes: Focus on Neurotransmitter Systems

In the following sections we will review the experiments done with transgenic, knock-out/in/down, and few conditional mice models targeting the main neurotransmitter and neuropeptide systems involved in the reinforcement and stress pathways. The findings obtained by the genetic manipulation within each particular system will be discussed separately.

2.1 Glutamate System

The role of glutamate receptors in mediating various alcohol effects has so far been studied using six different conventional global knock-out models, with genetic alterations in the NMDA receptor subunit NR2A (Sakimura et al. 1995), the AMPA subunits GluR1 (Zamanillo et al. 1999) and GluR3 (Sanchis-Segura et al. 2006), the metabotropic receptor subunits mGluR4 (Pekhletski et al. 1996), mGluR5 (Lu et al. 1997), and mGluR7 (Masugi et al. 1999; Vadasz et al. 2007). Very recently, the first conditional knock-out of the NR2B receptor subunit in the forebrain neurons has been developed and tested for alcohol-related behaviors (Badanich et al. 2011) (Table 2).

Initial sensitivity to alcohol has been shown to be barely affected by deletions of NMDA or AMPA receptor subunits. Thus, NR2A mutants showed no altered phenotype when tested for LORR or other acute responses to alcohol; home cage alcohol intake was also not different from wild-type mice (Boyce-Rustayand Holmes 2005, 2006; Sato et al. 2006; Palachick et al. 2008). Although the mice show no alterations in voluntary alcohol intake, the rewarding effects of alcohol, as measured by the CPP paradigm are strongly impaired (Boyce-Rustayand Holmes 2006). This provides one further example that CPP measures (where secondary reinforcement is measured) cannot be directly compared with home cage or operant drinking data where primary reinforcement is measured. In contrast to NR2A, forebrain deletion of the NR2B subunit increases the sensitivity to both the intoxicating and stimulating doses of alcohol (Badanich et al. 2011), indicating that NR2B convey the sensitivity to the acute actions of alcohol. This conclusion is further supported by findings in post-synaptic density 95 (PSD-95) knock-outs (Yao et al. 2004). PSD-95 is a key orchestrator of NMDA receptors and glutamatergic synapses and PSD-95 knock-outs exhibit increased sensitivity to the hypnotic effects of ethanol (LORR measures) and decreased alcohol intake (Camp et al. 2011). The AMPA GluR1 and GluR3 subunits neither play a role in initial alcohol sensitivity nor in alcohol intake (Palachick et al. 2008; Cowen et al. 2003; Sanchis-Segura et al. 2006). Similarly, mGluR4 knock-outs showed no altered phenotype in LORR or alcohol intake, except for a lack of alcohol-induced increase in locomotor activity (Blednov et al. 2004). In contrast, the altered alcohol phenotype in the mGluR5 knock-out mice has been widely supported by many studies. Thus, these mice displayed increased sensitivity to the intoxicating and rewarding effects of alcohol (Bird et al. 2008; Blednov and Harris 2008; Downing et al. 2010), with a not very clear phenotype in voluntary alcohol intake, due to contradictory findings reporting no alteration or avoidance (Blednov et al. 2004; Blednov and Harris 2008; Bird et al. 2008). Finally, mGluR7 knock-outs express increased alcohol consumption and mice carrying a mGluR7 variant with higher mGluR7 mRNA drink less alcohol (Gyetvai et al. 2011). These findings are however, in contrast with recent pharmacological data using the mGluR7 agonist AMN082 showing strongly reduced alcohol consumption and preference (Bahi 2011)—the reason for this discrepancy of results is at the moment unclear. In line

Table 2 Glutamate system

ADDICTION CYCLE	Site	Forebrain	Global										
	Type	Cre/loxP	KO						OE	KO			
	Target	NR2B	NR2A	GluR1	GluR3	mGluR4	mGluR5	mGluR7		PSD-95	Hyperglutamatergia ENT1	Per2	Glast
Initiation	LORR	↑	—	—		—	↑			↑	↓	↑	—
	CPP		↓				↑			—	—		↓
	Homecage		—	—	—	—	↓—	↑	↓	↓	↑	↑	↓
	Operant				—						↑	↑	
Maintenance	Chronic			—	—		↓			↓	↑	↑	
	Stress/Dependence			—/									
Craving & Relapse	Seeking				↓								
	Relapse			—	↓								
	Withdrawal					—	—	↑					
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)										CREB		
	Electrophysiological (Transmission)		—								↑		
	Biochemical (Release)										↑	↑	—

"OE": Congenic strain which carries the hyperfunctioning allelic variant
"Forebrain": CaMKII promoter

with the results reported during initiation of alcohol intake, long-term drinking was not altered in mice lacking the AMPA receptor subunit GluR1 (Cowen et al. 2003) and GluR3 (Sanchis-Segura et al. 2006) respectively, but was decreased in mGluR5 mutants (Bird et al. 2008). Alcohol-seeking and relapse has been shown to be specifically mediated by the AMPA receptor subunit GluR3. Thus, while relapse-like drinking is not altered in the AMPA subunit GluR1 receptor mutants (Cowen et al. 2003), GluR3 knock-out mice show a blunted cue-induced reinstatement response and a lack of an alcohol deprivation effect (ADE) despite normal operant and voluntary self-administration (Sanchis-Segura et al. 2006).

We also included in this Sect. 3 hyperglutamatergic mutant models. In the first two models either the period gene 2 (Per2) (Zheng et al. 1999) or the adenosine transporter ENT1 (Choi et al. 2004) is targeted leading to an indirect down-regulation of the glutamate transporters EAAT1 (in the case of Per2) and EAAT2, respectively (Spanagel et al. 2005; Wu et al. 2010; Nam et al. 2011). The third model targets directly the glutamate transporter EAAT1 also known as GLAST (Watase et al. 1998).

An induction of a hyperglutamatergic state is strongly affecting initial responses to alcohol. Thus, deletion of ENT1 or Per2, which leads to a down-regulation of the glutamate transporters EAAT2 and EATT1, respectively, induced opposing responses to an acute intoxicating challenge of alcohol, with decreased sensitivity in the ENT1(Choi et al. 2004; Chen et al. 2010) and increased sensitivity in the Per2 (Perreau-Lenz et al. 2009) mutants. However, both show identical phenotypes of increased alcohol intake and motivation, as tested by home cage or operant conditions (Choi et al. 2004; Chen et al. 2010; Nam et al. 2011; Spanagel et al. 2005). Indeed, the excessive intake in both models was also shown to be reduced by acamprosate administration. Acamprosate is known to act as an anti-hyperglutamatergic compound in the rodent (Spanagel and Kiefer 2008) and human brain (Umhau et al. 2010), supporting a hyperglutamatergic state mediated response in these mutant mouse models (Lee et al. 2011; Spanagel et al. 2005). This idea is further supported in recent experiments done in the ENT1 knock-outs where enhanced glutamate levels after acute alcohol injections occur (Chen et al. 2010; Nam et al. 2010), whereas ENT1 regulates alcohol drinking through accumbal NMDA-receptor signaling (Nam et al. 2011). Excessive glutamate also augments withdrawal seizures as has been demonstrated in ENT1 knock-out mice (Kim et al. 2011). Surprisingly, EAAT1 mutation (GLAST knock-outs) results in the opposite phenotype compared to Per2 knock-outs, as shown by the lack of alterations in the LORR test, and decreased rewarding effects of alcohol and decreased alcohol consumption—the discrepancy of results can however, be explained by strong developmental compensation mechanisms in GLAST knock-outs (Karlsson et al. 2012).

In summary, the results obtained with the above described mutants point to a minor role of the glutamate NMDA and AMPA receptors during the initiation to alcohol consumption, whereas the mGluR5 subunit seems to be involved in mediating not only initial alcohol sensitivity, but also reward sensitivity. On the other hand, the AMPA receptor subunit GluR3 might be a key mediator in craving and relapse responses. However, other mediators cannot be excluded as seeking,

relapse, and withdrawal have not been tested in most of the mutants. Especially the NR1 subunit within the mesolimbic system might be of great importance for the persistence of alcohol-seeking and relapse behavior; this has at least been shown in a conditional mouse mutant model for other drugs of abuse (Engblom et al. 2008; Mameli et al. 2009); these mice should be tested as well for phenotypes relevant for alcohol addiction. Finally, induction of a hyperglutamatergic state produces the most prominent phenotype, affecting the initiation, maintenance, and probably craving and relapse in alcohol-dependent animals.

2.2 GABA System

The importance of the GABA system in mediating various effects of ethanol has led to the development of a great number of mutants, generated by different genetic technologies. Thus, alcohol studies have been performed in 17 genetically manipulated mouse models targeting 11 different components of the GABAergic system (Table 3). All these mouse models have been tested for initial sensitivity to alcohol, and some of them have been further characterized for the initiation of alcohol consumption and withdrawal (Tables 3 and 4).

Acute sensitivity to alcohol has been shown to depend on specific subunit composition of α1, 2, and β2 receptors. Thus, while a first study reported decreased LORR in α1 subunit knock-outs (Blednov et al. 2003b), later studies demonstrated no alteration in these mutants (Kralic et al. 2003; Werner et al. 2006). Other responses are also mediated by this subunit, as reported by studies showing alterations in alcohol-induced locomotor stimulation (Blednov et al. 2003b; Kralic et al. 2003; June et al. 2007), gene expression (Harris et al. 2011) and GABA synaptic transmission (Werner et al. 2006).

Other required subunits for mediating the acute intoxicating effects of alcohol are the α2 and β2 subunits. Two independent studies reported decreased LORR responses after deletion of α2 and β2 subunits (Boehm et al. 2004; Blednov et al. 2003b). However, later, an increased sensitivity in the LORR test was demonstrated in α2 knock-outs, with no difference in the anxiolytic or motor incoordinating effects of alcohol (Blednov et al. 2011). Furthermore, activity-sensitive Arc and Fos transcripts were also increased after alcohol administration in these mice (Harris et al. 2011) In contrast, deletion of the 4 and 6 subunits, despite not affecting at a behavioral level (Chandra et al. 2008; Iyer et al. 2011), appears to regulate GABA synaptic neurotransmission (Liang et al. 2008; Suryanarayanan et al. 2011).

The genetic deletion of the GABA transporter GAT1 is likely another important modulator in mediating the acute action of alcohol. Thus, GAT1 mutants show decreased sensitivity to sedative doses of alcohol with increased tolerance, and higher sensitivity to the motor stimulant effect of alcohol (Hu et al. 2004; Cai et al. 2006). Intriguingly, overexpression of GAT1 also resulted in low sensitivity to alcohol, as shown by the righting reflex test (Hu et al. 2004). Other subunits tested

Table 3 GABA receptor subunit mutants

Target	Model	References
Alpha 1	KO	Vicini et al. (2001)
		Sur et al. (2001)
	KI	Borghese et al. (2006)
Alpha 2	KI	Werner et al. (2011)
Alpha 4	KO	Chandra et al. (2006)
Alpha 5	KO	Collison et al. (2002)
Alpha 6	KO	Jones et al. (1997)
Beta 2	KO	Sur et al. (2001)
Beta 3	KO	Homanics et al. (1997)
	KI	Jurd et al. (2002)
	Conditional	Ferguson et al. (2007)
Gamma	KO	Homanics et al. (1999)
	KD	Chandra et al. (2005)
	Overexpression	Wick et al. (2000)
Delta	KO	Mihalek et al. (1999)
GAT	KO	Cai et al. (2006)
	Overexpression	Ma et al. (2001)
Gad2	KO	Kash et al. (1997)

have shown the lack of mediation in the acute intoxication of alcohol. In this regard, it is worth to mention that the only conditional model tested in alcohol, the forebrain specific β3 subunit mutant (Ferguson et al. 2007), has supported the lack of phenotype previously reported in the global inactivation (Quinlan et al. 1998). Similarly, γ or δ subunits are not required for the alcohol's modulatory actions, as reported by no alterations in the alcohol-induced potentiation of GABA currents and several behavioral responses in mice with deletion of these subunit (Homanics et al. 1999; Berry et al. 2009; Shannon et al. 2004). Initial reinforcement processes have been much less studied in these models. Two studies showed an important role for the α1 subunit receptor in the consummatory and motivational properties of alcohol, as demonstrated by decreased alcohol self-administration under both home cage and operant paradigms, but normal CPP in α1 mutant mice (Blednov et al. 2003; June et al. 2007); however, another study reported no differences in voluntary alcohol consumption in these mice (Werner et al. 2006). Knock-in mice for the α2 subunit showed changes in a range of alcohol intake and preference tests, and did not develop the typical conditioned taste aversion in response to alcohol (Blednov et al. 2011). Single studies showed decreased voluntary, but not operant self-administration in α5 mutants (Stephens et al. 2005), reduced alcohol consumption in δ knock-out mice (Mihalek et al. 2001), and normal alcohol intake in β2 knock-outs (Blednov et al. 2003). Lastly, impairment of GABA synthesis by deletion of Gad2, increased non limited alcohol intake, in a background-dependent manner (Blednov et al. 2010), while deletion of the GABA transporter GAT1 did not affect this response, though it decreased alcohol aversion and reward, as measured by CTA and CPP (Cai et al. 2006). Maintenance of long-term drinking

Table 4 GABA system

ADDICTION CYCLE	Site	Global							Forebrain	Global					
	Type	KO/KI/KD							Cre/loxP	KO/KD	Transg.+	KO		Transg.+	KO
	Target	α1	α2	α4	α5	α6	β2	β3		γ		δ	GAT		Gad2
Initiation	LORR	↓–	↑	–	–	–	↓	–	–	–	–	–	↓	↓	–
	CPP	–					–						↓		
	Homecage	↓–	↓–		↓		–					↓	–		↑–
	Operant	↓			–										
Maintenance	Chronic		↓				–								
	Stress/Dependence														
Craving & Relapse	Seeking														
	Relapse														
	Withdrawal	↑–				–	–	↑		–	–	↓			
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)	Arc,Fos	Arc,Fos												
	Electrophysiological (Transmission)	↓		↑						–					
	Biochemical (Release)														

"Forebrain": CaMKII promoter

has been shown to be decreased in α2 and, as observed during acquisition phase, not altered in β2 mutant mice (Blednov et al. 2011, 2003).There are almost no studies on the role of different GABA subunits in alcohol craving and relapse. Indeed, these studies have only focused on the withdrawal severity from alcohol, measured by HIC. They have shown contrasting results regarding α1 subunit, showing no alterations or increased HIC (Blednov et al. 2003; Werner et al. 2009), while the α6 subunit exerts little if any influence on withdrawal hyperexcitability Homanics et al. 1998). β3 and δ subunit deletions, however, appear to participate in alcohol withdrawal in opposite ways, as demonstrated by the increased and decreased HIC scores shown in β3 and δ mutants, respectively (Sanchis-Segura et al. 2007; Blednov et al. 2003; Mihalek et al. 2001). β2 or γ (both deletion or overexpression) do not seem to mediate these responses (Blednov et al. 2003; Homanics et al. 1999; Wick et al. 2000).

In summary, these studies support the importance of $GABA_A$ receptors in mediating behavioral actions of alcohol. Thus, while specific α subunits have a modulatory role in the initiation of alcohol consumption, others, like the β2 subunit or δ receptor, might become gradually more important in mediating motivational aspects of alcohol and withdrawal. On the other hand, GABA transporter or synthesis deletions also might have a role during this first phase. Unfortunately, other components of the GABA system have not been studied in alcohol (like other $GABA_A$ subunits, or the $GABA_B$ receptor). In addition, the potential influence of these alterations in later stages of alcohol dependence (craving and relapse) remains unexplored.

2.3 Dopamine and Serotonin Systems

The consequences of genetic manipulation of the dopamine system in alcohol addiction has been demonstrated with the use of four models targeting the complete deletion of the D1 (Drago et al. 1994), D2 (Kelly et al. 1997, 1998; Wang et al. 2000), D3 (Xu et al. 1997; Steiner et al. 1997) and D4 (Rubinstein et al. 1997) receptors, and the dopamine transporter DAT (Giros et al. 1996; Sora et al. 1998). Much of the work with these models has been focused on the initiation phase of alcohol consumption, with almost no studies developed during the maintenance or craving and relapse phases (Table 5).

Initial effects of alcohol in D1 receptor knock-outs have only been tested under voluntary alcohol drinking, where they show decreased consumption and preference either during limited, continuous, or forced exposure (El-Ghundi et al. 1998). On the other hand, the mice do not show acute nor sensitized locomotor responses to alcohol (Harrison and Nobrega 2009). D2 receptor mutants, better characterized during this phase, show decreased alcohol intake both under home cage and operant paradigms, and decreased CPP (Phillips et al. 1998; Risinger et al. 2000; Cunningham et al. 2000; Thanos et al. 2005a; Ting-A-Kee et al. 2009), with only one study reporting no differences in home cage at high and forced alcohol (20%)

Table 5 DA and 5HT system

ADDICTION CYCLE	Site	Global								Forebrain	Global			
	Type	KO								Transg.+	KO			
	Target	D1	D2	D2L	D3	D4	DAT	5HT1A	5HT1B	5HT3	5HT3A	5HT6	5HTT	Vmat2
Initiation	LORR				↑		—		↓	—		↓	↑	—
	CPP		↓		—				↓				—	↓
	Homecage	↓	↓—	↓	↓—	—	↑—		↑—	↓—	—	—	↓—	↑↓
	Operant		↓		—		↓		↑					
Maintenance	Chronic	↓	↓—										↓	
	Stress/Dependence													
Craving & Relapse	Seeking													
	Relapse													
	Withdrawal				↑				—					
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)		CB1											
	Electrophysiological (Transmission)									↑			↑	
	Biochemical (Release)		↓				—						—	

"Forebrain": CaMKII promoter

concentrations (Thanos et al. 2011).The role of the D2 receptor during this initial phase is supported by other behavioral and biochemical experiments showing reduced sensitivity to alcohol-induced ataxia, locomotor sensitization, or aversion (Phillips et al. 1998; Palmer et al. 2003; Ting-A-Kee et al. 2009), and reduced striatal dopamine overflow after acute alcohol challenge (Job et al. 2006) in D2 knock-out mice. Moreover, a very recent study points to the specific D2 receptor isoform D2S in modulating alcohol intake (Bulwa et al. 2011). D3 receptor knock-outs show higher sensitivity to alcohol, as shown by increased LORR duration after intoxication and decreased alcohol intake during forced exposure (Narita et al. 2002). However, free-choice home cage and operant self-administration, or place preference/aversion is not altered in the same mutants (Boyce-Rustay and Risinger 2003; McQuade et al. 2003). In spite of this apparent, "normal" phenotype during initial reinforcement processes, D3 receptor mutants show decreased alcohol metabolism and an absent acute or sensitized locomotor responses to alcohol (McQuade et al. 2003; Harrison and Nobrega 2009). A single study reported no alterations in D4 knock-outs in home cage drinking (Falzone et al. 2002), although previously it was shown increased alcohol locomotor stimulation in these mice (Rubinstein et al. 1997). The effect of a hyperdopaminergic state during the initiation of alcohol reinforcement processes, by the use of the DAT knock-out, has been contradictory. Thus, while the first study reported no alterations during intoxication or home cage drinking in DAT mutant mice (note: only the females showed altered responses) (Savelieva et al. 2002), the second study reported increased alcohol intake, but only at high (24 and 32%) concentrations (Hall et al. 2003). On the other hand, an acute challenge of alcohol induced a higher locomotor activating response in the mutants (Morice et al. 2010), while the striatal dopamine release was not affected (Mathews et al. 2006). The more recent study has not supported any of the findings previously reported, and suggests that dopamine dynamics are associated with alcohol consumption. Thus, the authors show that DAT knock-out mice consumed less alcohol under operant conditions, a phenotype related to differences in dopamine autoreceptor sensitivity, DAT efficiency, and DAT capacity (Mittleman et al. 2011). The number of studies testing the maintenance of chronic, long-term drinking has been very limited. However, they support the findings already obtained during the initiation phase with the D1 (Short et al. 2006) and D2 (Risinger et al. 2000; Palmer et al. 2003; Thanos et al. 2005a; Thanos et al. 2011) receptor mutants. Furthermore, an endocannabinoid system-dependent interaction has been proposed to explain the decreased alcohol intake shown by D2 mutants, as demonstrated by the increased endocannabinoid signaling occurring in these mice, and its reversal by chronic alcohol intake (Thanos et al. 2011). Even less is known about the phenotype of these models during craving and relapse. On one hand, two independent studies point to a possible role of D2 in alcohol-mediated long-term neuroadaptations, as shown by the findings that, the differences in alcohol drinking and CPP observed in D2 mutants disappear when the animals have been pretreated with alcohol or withdrawn from an alcohol liquid diet (Palmer et al. 2003; Ting-A-Kee et al. 2009). On the other hand, the role played by D3 receptor is not

clear, as one study reported a transient increase in the withdrawal score in D3 mutants (Narita et al. 2002).

There are six genetically altered mouse models targeting the serotonin system that have been used for alcohol experiments. These include the complete deletion of the 5HT1A (Ramboz et al. 1998), 5HT1B (Saudou et al. 1994; Ramboz et al. 1996), 5HT3A (Zeitz et al. 2002) and 5HTA (Bonasera et al. 2006) subunit receptors, and the serotonin transporter 5HTT (Bengel et al. 1998). A 5HT3 receptor overexpression exclusively in the mouse forebrain has also been characterized (Engel et al. 1998).Similar to the dopamine system, most of the work with these models has concentrated on the initiation of alcohol reinforcement processes.

Thus, sensitivity to high, intoxicating doses of alcohol assessed by LORR was decreased in 5HT1B (Boehm et al. 2000; Crabbe et al. 1996) and 5HT6 (Bonasera et al. 2006) mutants. 5HT1A knock-out, although not tested for LORR, showed decreased hypothermic effect to a sedative dose of alcohol (Pattij et al. 2002). In contrast, LORR is increased in 5HTT mutant mice (Daws et al. 2006; Boyce-Rustay et al. 2006). Paradoxically, in the same mice, alcohol-induced inhibition of 5HT clearance was potentiated, indicating a 5HTT independent mechanism in the behavioral response (Daws et al. 2006). On the other hand, overexpression of the 5HT3A subunit in the forebrain did not affect LORR, but increased both the behavioral sensitivity to stimulatory doses of alcohol (Engel and Allan 1999) and synaptic responses of 5HT3 receptors (Sung et al. 2000). Surprisingly, the global 5HT3 mutants did not show alterations in alcohol-induced locomotor activation (Hodge et al. 2004). The role of these receptors on reinforcement is less clear, due to the frequent contradictory results obtained. The studies using the 5HT1B mutant represent an excellent example. Thus, from the first publication, showing increased voluntary alcohol intake in 5HT1B receptor mutants (Crabbe et al. 1996), the same author reported 3 years later a lack of phenotype, a conclusion supported by three different labs (Crabbe et al. 1999). After that, two more studies replicated those results, though in the last one the tendency toward increased intake was again manifested (Bouwknecht et al. 2000; Gorwood et al. 2002). Besides that, operant self-administration was increased with an unsweetened alcohol solution (Risinger et al. 1999). The CPP response was also shown to be impaired; a finding that could result from the lack of any locomotor activity increase during the conditioning phase (Risinger et al. 1996). Similar to 5HT1B mutants, 5HT3A and 5HT6 knock-out mice did not show alterations in alcohol intake (Hodge et al. 2004; Bonasera et al. 2006). 5HT3A overexpressing mice showed a decreased, but strain-specific, alcohol intake (Engel et al. 1998; Metz et al. 2006), with no alterations in the motivation to work for alcohol, as shown during operant self-administration (McKenzie-Quirk et al. 2005) or in alcohol discriminative stimulus effects (Shelton et al. 2004). In contrast, deletion of the 5HTT appears to modulate alcohol reinforcement. Thus, evidences from two independent studies done in 5HTT mutants, have found decreased CPP response and decreased alcohol intake, shown during the 24-h period, or during the peak period of drinking in the early dark phase (Kela et al. 2003; Boyce-Rustay et al. 2006). Furthermore, this same

phenotype was also observed during long-term drinking (Kela et al. 2003). Craving and relapse have only been tested in 5HT1B knock-outs by means of withdrawal symptoms after alcohol vapor exposure, which were not altered in these mice (Crabbe et al. 1996). Lastly, few studies have shown the contribution of the vesicular monoamine transporter Vmat2 deletion (Wang et al. 1997) on alcohol-related phenotypes. These studies have shown no alterations in alcohol intoxication (Savelieva et al. 2006), but increased sensitivity to low, stimulatory doses of alcohol (Wang et al. 1997). The question about the role of Vmat2 in alcohol intake is still unresolved, as so far the only two studies using Vmat2 mutants have shown opposite phenotypes, and lack of alcohol CPP (Hall et al. 2003; Savelieva et al. 2006).

In summary, the relative contribution of specific components of the dopamine and serotonin systems during the different phases of alcohol addiction has been focused on the initial reinforcement processes. Thus, the results point to a more prominent role of the dopamine D1 and D2 receptors, and the serotonin 5HT1B receptor subunit and 5HTT transporter during the initiation of alcohol consumption. However, one should keep in mind that not all the mutant models have been tested completely during this initiation phase; and craving and relapse remain to be further explored in almost all mutant models.

2.4 Endocannabinoid System

Considering the extensive literature supporting the key role of the ECS in alcoholism (Rodríguez de Fonseca et al. 2005), it is surprising the low number of studies addressing the effects of alcohol on this system by the use of genetically modified mouse models. Thus, only three global mutant models, namely, the cannabinoid CB1 (Zimmer et al. 1999) and CB2 (Buckley et al. 2000) receptors, and the endogenous endocannabinoid AEA degradative enzyme FAAH (Cravatt et al. 2001) have been studied in alcohol-related phenotypes (Table 6).

The few number of studies testing the influence of the CB1 receptor deletion in the initial sensitivity to alcohol are not always very conclusive, due to the contradictory results obtained in some tests. Nevertheless, what can be concluded is the mediation of CB1 receptor in the hypnotic/sedative effect of alcohol, as LORR has been shown to be increased in CB1 knock-out mice in two independent studies (Vinod et al. 2008a; Naassila et al. 2004). The role of CB1 in mediating the hypothermic response to alcohol is less clear, as it has been reported not to be altered (Racz et al. 2003), increased (Naassila et al. 2004; Warnault et al. 2007) or even decreased (Vinod et al. 2008a) in CB1 knock-out mice. The increased sensitivity displayed by the CB1 mutants can be a protective factor contributing to decreased alcohol intake and reinforcement. In agreement with this idea, several studies have found decreased voluntary alcohol intake and/or preference, as measured by home cage drinking and CPP (Lallemand and De Witte 2005; Wang et al. 2003; Poncelet et al. 2003; Hungund et al. 2003; Naassila et al. 2004; Thanos

Table 6 ECS

ADDICTION CYCLE		Site: Global		
		Type: KO		
	Target	CB1	CB2	FAAH
Initiation	LORR	↑		↓—
	CPP	↓		—
	Homecage	↓—		↑—
	Operant			
Maintenance	Chronic	↓—	—	
	Stress/Dependence	↓/↓		
Craving & Relapse	Seeking			
	Relapse			
	Withdrawal	↕		↓—
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)	NMDA GABA		
	Electrophysiological (Transmission)			
	Biochemical (Release)	↓*		

*"Dopamine"

et al. 2005b; Vinod et al. 2008a; Houchi et al. 2005), with a single study reporting no alterations (Racz et al. 2003). In line with these findings, an increase in the endogenous endocannabinoid tone, by deleting AEA degradation, leads to the opposite phenotype. Thus, FAAH knock-out mice are less sensitive to the alcohol intoxicating, hypothermic, and sedative effects compared to control mice, (Vinod et al. 2008b; Blednov et al. 2007), though, again, contradictory results can also be found (Basavarajappa et al. 2006). Furthermore, FAAH mutants also show increased alcohol intake and preference (Vinod et al. 2008b; Blednov et al. 2007). However, this phenotype might be indirectly modulated by FAAH (and thus, increased AEA levels), since these mice show the expected decrease in CB1 receptor number and affinity after voluntary alcohol consumption (Basavarajappa et al. 2006). Another discrepancy is found in the lack of any phenotypic alteration in developing a place preference for alcohol (Blednov et al. 2007). Other acute effects, like alcohol-induced anxiolytic responses or ataxia, are not affected in CB1 or FAAH knock-outs (Houchi et al. 2005; Racz et al. 2003; Blednov et al. 2007). Maintenance of long-term drinking as well as stress- or dependency-induced excessive drinking is similarly decreased in CB1 knock-outs (Racz et al. 2003;

Lallemand and De Witte 2005; Warnault et al. 2007). Furthermore, a motivational-based mechanism has been proposed to be mediating this phenotype. Thus, when exposed to forced alcohol drinking, and thereby increasing the motivational state of the mouse, differences in alcohol drinking are not observed any more (Lallemand and De Witte 2005; Warnault et al. 2007). Supporting this idea, the frequently observed age-dependent decline in alcohol preference and intake (due to a decreased motivational state) is absent in CB1 knock-out mice, leading to equal and indistinguishable amounts of alcohol intake among old CB1, CB2 receptor knock-outs, and wild-type mice (Wang et al. 2003; Trebicka et al. 2011). These data strongly indicate that a decrease in CB1 receptor activity might correlate with decreased activation of reward-dependent pathways. A possible mechanism for the decreased reward sensitivity observed at the behavioral level might involve glutamatergic and GABAergic neurotransmission. Thus, NMDA and GABA neuroadaptations induced by chronic alcohol administration are absent in CB1 mutants, besides other basal neuroadaptaions (Warnault et al. 2007). Furthermore, the neurotoxic effect of alcohol in the immature brain, which is also mediated by glutamate and GABA transmission, is attenuated in CB1 knock-out infants (Hansen et al. 2008). Interestingly, the low behavioral and molecular alcohol sensitivity displayed by the CB1 mutant mouse, appears not to be restricted to the CNS. Thus, a very recent study has demonstrated an attenuated liver damage after long-term chronic alcohol administration in CB1 knock-outs, while this effect was more pronounced in CB2 deficient mice (Trebicka et al. 2011). There is little and inconclusive evidence demonstrating the role of the ECS in craving and relapse using CB1 and FAAH mutants. These studies have reported both decreased and increased withdrawal after forced drinking or dependence induction in CB1 knock-outs (Racz et al. 2003; Vinod et al. 2008a; Naassila et al. 2004). A similar contrasting picture can be found in studies using the FAAH knock-outs, showing either reduced (Vinod et al. 2008b) or not altered (Blednov et al. 2007) withdrawal scores.

In summary, CB1 deletion results in increased initial sensitivity to alcohol that is associated with decreased alcohol intake and preference, with no clear function during withdrawal states. However, its role in some alcohol-related behaviors remains unclear, like craving, alcohol-seeking behavior, and relapse. In addition, other components of the ECS, as FAAH, CB2 receptor, or other endocannabinoids that might also be mediating some aspects of alcohol intake, have been barely explored.

2.5 Opioid System

Among the opioid receptor knock-outs, the μ-opioid receptor mutant was the first developed and the most studied in the actions of alcohol. In fact, three models were generated using different technologies of insertion, deletion, or replacement (Matthes et al. 1996; Sora et al. 1997; Loh et al. 1998). Delta (δ) (Filliol et al.

Table 7 Opioid system

ADDICTION CYCLE	Site	Global					
	Type	KO					
	Target	μ	δ	κ	β-end	Penk	Dyn
Initiation	LORR						—
	CPP	—				—	↑—
	Homecage	↓—	↑—	↓	↕	—	↑—
	Operant	↓	—		↑—	—	
Maintenance	Chronic	↓	↑	↓	—	—	—
	Stress/Dependence				↓/	↓/	↓/
Craving & Relapse	Seeking						
	Relapse				↑		
	Withdrawal	↑			—		—
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)						TH, δ,μ, κ
	Electrophysiological (Transmission)	—**	↑**				
	Biochemical (Release)	↓—*		↑*			

*"Dopamine"
** "GABA"

2000) and kappa (κ) (Simonin et al. 1998) receptor mutants, and knock-out for the endogenous peptides β-endorphin (Rubinstein et al. 1996), proenkephalin (König et al. 1996), and prodynorphin (Sharifi et al. 2001) have also been tested, although not so extensively, in respect to alcohol-related phenotypes (Table 7).

Initial sensitivity to the intoxicating effects or other behavioral responses of alcohol have not been studied in any of the receptor mutants, except for the μ-opioid receptor knock-out, where opposing phenotypes have been found in both the locomotor stimulant and anxiolytic effects of alcohol (Filliol et al. 2000; LaBuda and Fuchs 2001; Hall et al. 2001; Ghozland et al. 2005). This lack of agreement on the results can also be oberverd in the experiments examining the initiation to alcohol intake and reinforcement processes not only in μ-, but also in δ-opioid receptor receptor mutants. Thus, several studies have reported no phenotype alterations in μ-opioid receptor knock-outs during voluntary, home cage drinking or CPP (Hall et al. 2001; Becker et al. 2002; Van Rijn and Whistler 2009), with a single one reporting decreased voluntary and operant alcohol intake (Roberts et al. 2000). Supporting the lack of behavioral phenotype, biochemical, molecular, and electrophysiological measures do not provide evidence on the

involvement of μ-opioid receptors in initial responses to alcohol. Hence, acute alcohol-induced accumbal dopamine release (Ramachandra et al. 2011, but Job et al. 2007), FOS activity (Kolodziejska-Akiyama et al. 2005) or GABA synaptic responses (Kang-Park et al. 2009) in the knock-outs do not differ from wild-type controls. Neither were the results obtained in alcohol intake with the δ-opioid receptor knock-out mice very clear, as it has been reported either increased (Van Rijn and Whistler 2009), or not altered acquisition of home cage or operant self-administration (Roberts et al. 2001) However, GABAergic synaptic sensitivity to alcohol in the central nucleus of the amygdale was increased in these mutants (Kang-Park et al. 2007). In contrast to the μ and δ, the less studied phenotype of the κ receptor mutant suggests a potential role in the modulation of alcohol. Thus, genetic deletion of κ receptor leads to decreased voluntary alcohol intake, a phenotype that could result from disrupted taste preferences (Kovacs et al. 2005; Van Rijn and Whistler 2009); however, this phenotype could also be linked to the reported increased sensitivity to the acute alcohol-induced dopamine release in the nucleus accumbens (Zapata and Shippenberg 2006). In contrast to the results obtained with the opioid receptor mutants, endogenous peptide deletions appear not to have an important role in the initial effects of alcohol. Thus, β-endorphin knock-out mice show altered alcohol-related phenotypes with certain environmental stress load. Acutely, alcohol strongly reduces their anxiogenic phenotype, even at lower levels than it does in the wild-type mice (Grisel et al. 2008), clearly indicating increased sensitivity to the anxiolytic effect of alcohol. In line with the hypothesis of increased sensitivity, in some conditions, this mutant shows increased voluntary alcohol intake such as at lower concentrations (Grisel et al. 1999) or during limited access (Grahame et al. 2000), though others have reported decreased intake, (Racz et al. 2008). Surprisingly, operant self-administration has been reported not to be altered (Grahame et al. 1998; Hayward et al. 2004). In contrast, proenkephalin peptide mutants failed to show any phenotype during the initiation phase, as all of the studies performed have found alterations in alcohol-induced hypothermia or tolerance, alcohol consumption or reinforcement (Racz et al. 2008; Koenig and Olive 2002; Hayward et al. 2004). Similar to proenkephalin, the lack of mediation of dynorphin in the acute sensitivity to alcohol intoxication has been clearly shown by two studies (Blednov et al. 2006; Femenía and Manzanares 2011). However, alcohol intake and reinforcement has shown to be not altered (Blednov et al. 2006; Sperling et al. 2010) or increased (Femenía and Manzanares 2011). Interestingly, the later study also reported alterations in these mice in opioid and dopamine gene expression in the dopaminergic reinforcement system, suggesting a link with the vulnerability for alcohol consumption. In contrast to the initiation, maintenance of chronic consumption of alcohol appears to be strongly mediated by the opioid system. Thus, long-term home cage alcohol intake was decreased in μ- and κ-opioid receptor knock-outs (Becker et al. 2002; Kovacs et al. 2005). In δ mutants, home cage drinking was only increased after an experience of operant self-administration. Indeed, chronic operant self-administration was also increased (Roberts et al. 2001). Experiments with endogenous peptides mutants have clearly demonstrated a major role in

stress-induced excessive drinking. Thus, long-term drinking experiments have demonstrated that β-endorphin, proenkephalin, and prodynorphin mutants lack any stress-induced increase in voluntary alcohol intake, despite no alterations in basal intake (Racz et al. 2008; Sperling et al. 2010). There is not much information about the role of the opioid system during craving and relapse. The only receptor mutant tested, the μ knock-out mouse, shows earlier withdrawal symptoms, suggesting accelerated progression of dependence (Ghozland et al. 2005). In the same direction, deletion of the endogenous ligand for this receptor, prodynorphin, leads to a slight, not significant increase in withdrawal (Racz et al. 2008), and relapse appears to be increased, as manifested by a kind of ADE, tested after 2 days of deprivation during limited access, that is, not showing up in the wild types (Grahame et al. 2000). Prodynorphin mutants have shown no alterations in withdrawal, as measured by HIC (Blednov et al. 2006; Femenía and Manzanares 2011).

In summary, the data obtained with these mutant models indicate a major role of the opioid system during long-term alcohol drinking, specially linked to environmental stress interactions, rather than during the initiation phase. However, most of the work has focused in the study of few models (μ receptor and β-endorphin mutants), and further characterization of other knock-outs could help to get a clearer picture. Moreover, the fact that none of the receptor mutants show any overlapping responses with any peptide partner mutants, strongly suggest compensatory neuroadaptations.

2.6 Corticotropin Releasing Factor System

A growing body of evidence points to a role of the corticotropin releasing factor system (CRF) in excessive drinking and alcohol dependence, a phenomenon hypothesized to be mediated by long-term up-regulation of CRF1 receptors in the amygdale (Heilig and Koob 2007). Indeed, six genetically modified models have been used to study the role of this system in alcohol-related phenotypes. These models include two global CRF1 receptor mutants, developed by two different labs (Smith et al. 1998; Timpl et al. 1998), one conditional brain-specific CRF1 receptor mutant (and thus, extra HPA axis deletion, Schmidt et al. 2006), one CRF2 receptor mutant (Coste et al. 2000), and two CRF models: one deleted (Muglia et al. 1995) and one over-expressed (Stenzel-Poore et al. 1992) (Table 8).

Studies testing the role of the CRF system in the initial, acute sensitivity to alcohol have indicated a regulatory role manifested under a constitutive hyperactivated system. Thus, the sedative effect of alcohol measured by the LORR test is not affected by CRF1, CRF2 receptors, or CRF deletions (Pastor et al. 2011; Sharpe et al. 2005; Olive et al. 2003), but increased only when CRF is over-expressed (Palmer et al. 2004). However, a modulation by CRF1 receptor in other alcohol-related effects cannot be excluded, such acute locomotor stimulating effect of alcohol or binge intake, using the DID protocol, where it was shown to be decreased in CRF and CRF1 knock-outs, and intact in CRF2 mutants (Olive et al.

Table 8 CRF system

ADDICTION CYCLE		Site	CNS	Global			
		Type	Cre/loxP	KO			Transg.+
		Target	CRF1		CRF2	CRF	
Initiation	LORR			—	—	—	↑
	CPP					↓	
	Homecage	—	↓—	—	↑	↓
	Operant						
Maintenance	Chronic	—	—			↓
	Stress/Dependence	↓/↓	↕/↕			
Craving & Relapse	Seeking					
	Relapse	—	—			
	Withdrawal		↓			
Other Alcohol-related Phenotypes	Molecular (Signal Pathway)					
	Electrophysiological (Transmission)		↓*			
	Biochemical (Release)					

"CNS": Nestin promoter

*"GABA"

2003; Pastor et al. 2008; Kaur et al. 2012). Furthermore, central and peripheral responses were also shown to be affected by CRF1 mutation. Thus, an acute alcohol challenge did not induce the expected increases either in GABA synaptic inhibition in the central nucleus of the amygdale (Nie et al. 2004), or in plasma ACTH and CORT levels (Lee et al. 2001) in CRF1 knock-outs. On the other hand, the CRF system does not seem to mediate the voluntary consummatory aspect of alcohol at moderate concentrations. When given access to alcohol in the home cage, CRF mutants consume more alcohol (Olive et al. 2003); however, CRF over-expressing mice show only partially the opposite phenotype, consuming less alcohol than the controls only at higher (20%) concentrations (Palmer et al. 2004). Indeed, this same phenotype of altered sensitivity to high (20%) but not lower (8%) alcohol concentration solutions has also been observed by several studies when CRF1 receptor is deleted (Pastor et al. 2011; Sillaber et al. 2002; Molander et al. 2011). Furthermore, this finding has been recently confirmed using an extra-hypothalamic conditional CRF1 receptor mouse model (Molander et al. 2011), supporting the idea of a central mediation of this phenotype. CRF2 receptor

mutants do not show any alteration in alcohol consumption (Sharpe et al. 2005). Instead, the CRF system has a particular, but still not completely elucidated role in excessive drinking, as demonstrated by the studies done with the CRF1 receptor knock-outs. Thus, the ability of stress to increase alcohol consumption has been reported to be increased or decreased in the CRF1 mutants compared to the wild types (Sillaber et al. 2002; Pastor et al. 2011; Molander et al. 2011). Interestingly, the excessive drinking has also shown to be absent when the HPA axis CRF1 receptors remain intact using a brain-specific conditional CRF1 knock-out mouse (Molander et al. 2011). On the other hand, in a post- dependent state, where animals have a history of dependence induced by alcohol vapor exposure, the findings are also confusing. In one study, CRF1 knock-out mice do not show any increase in operant alcohol self-administration following an induction of dependence (Chu et al. 2007), while in another study the opposite phenotype has been reported, showing an escalation effect (Molander et al. 2011). This last study additionally shows that this effect is absent in the global mutants, proposing a role for the HPA axis-containing CRF1 receptors in mediating opposing effects on stress-induced and post-dependent alcohol drinking (Molander et al. 2011). Only two studies have addressed the role of the CRF system in craving and reinstatement of alcohol-seeking. The first study reported decreased withdrawal symptoms from alcohol after forced drinking in CRF1 mutants (Timpl et al. 1998), while a more recent one shows no alterations in relapse, as measured by ADE, in global or CNS conditional CRF1 mutants (Molander et al. 2011). In summary, though the extensive evidence supporting a role for the CRF system in alcohol-related behaviors, especially excessive alcohol consumption, the contribution from the use of mutant mice is still inconclusive, due to the insufficient number of studies and the contradictory results obtained.

In summary, the valuable contribution from genetically engineered mouse models, providing evidence for the role of the reinforcement and stress pathways in different alcohol-related phenotypes, is nearly exclusively supported by studies using knock-out and transgenic models. These findings support the general conclusion that alcohol affects all neurotransmitter systems, as demonstrated by the more or less severely altered alcohol-induced responses in virtually all the genetic models tested. However, the contribution of a particular component system to each phase of the addiction cycle is extremely difficult to define with the use of conventional models of global deletions, limited by the lack of site or time specificity and the resulting developmental neuroadaptations that might interfere and strongly compromise alcohol-related phenotypes.

3 Future Directions: Advanced Genetic Models to Study Alcohol-Related Phenotypes

Considering the limitations mentioned in the previous section, there is a great deal of work to be done to ensure that a particular gene manipulation is responsible for the observed phenotype. The use of conditional mutants should overcome to a

certain degree these limitations. Over the past decade, many novel tools have been generated to alter gene function, in a more refined way, providing not only site, but also time-specific deletions. These tools include the conditional Cre/loxP, inducible CreERT2, and Tet-Off and Tet-On systems. In the Cre/loxP system, the gene ablation is restricted to a defined group of cells. This system uses the properties of the Cre recombinase, an enzyme derived from bacteriophage P1, which has the ability to cut and ligate DNA strands. Cre recognizes specific sequences, the loxP sites, which are not normally present in the murine genome. When a transgenic mouse harboring Cre under the control of a cell-type specific promoter is crossed with an animal that contains a gene containing loxP sequences, a deletion in the target gene will occur, but only in Cre-expressing cells. A major advance is the development of ligand-dependent Cre recombinases that can be activated by administration of tamoxifen to the animal, generating time- and tissue-specific mouse mutants, the so-called inducible CreERT2 lines. Lastly, in the Tet-Off and Tet-On system, the expression of the transcriptional activator can be regulated both reversibly and quantitatively by exposing the transgenic animals to varying concentrations of tetracycline (Tc), or Tc derivatives such as doxycycline (Dox). Unfortunately, as was already mentioned, the contribution from the use of conditional models to study alcohol-related phenotypes has been very low (Table 9).

In the following sections we will summarize the current findings obtained from conditional mouse models, and propose them for a potential use in alcohol research (Table 10).

3.1 Glutamate System

Conditional and inducible (time restricted) deletions of NR1, GluR1, and GluR2, respectively, in dopaminergic neurons are available. These mice, generated by the laboratory of Günter Schütz (Heidelberg, Germany) have been characterized in the appetitive memory formation, and do not show any of the behavioral abnormalities observed in the global mutants that could potentially mask the effects on alcohol-related behaviors (like basal increased activity, ataxia, or learning deficits). Using these mice it could be shown that dopamine-dependent motivational learning and extinction processes are regulated by NMDA and AMPA receptors (Engblom et al. 2008).

Metabotropic receptors have also been conditionally deleted. Thus, deletion of mGluR1 in cerebellum (Nakao et al. 2007) results in impaired motor coordination, suggesting that mGluR1 is essential for cerebellar function in mice, not only during postnatal development but also in adulthood. A second model has recently been developed also by the Schütz laboratory, which is a knockdown of the mGluR5 receptor in the dopaminoceptive neurons, and is critical for the recall of appetitive memories. Thus, in a very recent study we have demonstrated that knocking down mGluR5 in dopaminoceptive neurons alters incentive learning processes that contribute to recall of appetitive memories (Novak et al. 2010). Finally, several conditional mutants exist already for the glutamate transporter vGlut2 (for a review see

Table 9 The number of studies performed on phenotypes relates to the alcohol addiction cycle with global knockout and transgenic (**G**) or conditional (**C**) mouse models

System	Glutamate		GABA		Dopamine		Serotonin		EC		Opioid		CRF		TOTAL	
Mutation	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
Addiction Cycle																
INITIATION	21	1	27	1	24	0	20	3	12	0	23	0	8	1	141	6
MAINTENANCE	6	0	2	0	5	0	1	0	6	0	5	0	3	1	29	1
CRAVING & RELAPSE	5	0	7	0	3	0	1	0	4	0	5	0	1	1	27	1
TOTAL	23	1	27	1	25	0	20	3	15	0	25	0	11	1	144	6

Wallén-McKenzie et al. 2010). Furthermore, a very recent study has demonstrated a relevant role of this transporter in dopaminergic neurons for reward processes, as conditional deletion of vGlut2 in DAT containing neurons leads to perturbations of reward consumption as well as reward-associated memories (Alsiö et al. 2011).

Table 10 Potential advanced genetic tools available for alcohol research

SYSTEM	METHOD	PROMOTER	TARGET	MOUSE	TARGETED SITE	REFERENCE
Glutamate	CreERT2/loxP	DAT	NR1	NR1^{DATCreERT2}	Midbrain DA neurons	Engblom et al., 2008
	CreERT2/loxP	DAT	GluR1	GluR1^{DATCreERT2}	Midbrain DA neurons	Engblom et al., 2008
	CreERT2/loxP	DAT	GluR2	GluR2^{DATCreERT2}	Midbrain DA neurons	Engblom et al., 2008
	Transgene	D1	miR-mGluR5	mGluR5^{D1-KD}	Striatal D1-MSNs	Novak et al., 2010
	Tta/TRE	L7	mGluR1	L7-mGluR1	Cerebellum	Nakao et al., 2007
	Cre/loxP	SF1	VGlut2	VGlut2$^{SF-1Cre}$	Ventromedial hypothalamus	Tong et al., 2007
	Cre/loxP	CamKII	VGlut2	VGlut2^{CamKIICre}	Cortex, amygdala	Wallen-McKenzie et al., 2009
	Cre/loxP	DAT	VGlut2	VGlut2^{DATCre}	Midbrain DA neurons	Birgner et al., 2010
GABA	Cre/loxP	CamKII	Alpha1	Alpha1^{CamKIICre}	Hippocampus, cortex, amygdala	Sonner et al., 2005
	Cre/loxP	Thy1	Alpha6	Alpha 6^{ThyCre}	Hippocampal CA1 neurons	Wisden et al., 2008
	Cre/loxP	Cre deleter	$GABA_B$	GABAB$^{-/-}$	CNS	Haller et al., 2004
Serotonin	Cre/loxP	Sert	Vmat2	Vmat2^{SertCre}	Raphe neurons	Nabous-Neme et al., 2011
Endocannabinoid	Cre/loxP	CamKII	CB1	CB1^{CamKIICre}	Cortical glutamate neurons, striatum, thalamus, hypothalamus	Marsicano et al., 2003
	Cre/loxP	Dlx5/6	CB1	CB1$^{Dlx5/6Cre}$	GABA interneurons	Monory et al., 2006
	Cre/loxP	NEX	CB1	CB1^{NEXCre}	Cortical glutamate neurons	Kepplisch et al., 2003
	Cre/loxP	D1	CB1	CB1^{D1Cre}	Striatal D1-MSNs	Monory et al., 2007
	Cre/loxP	Sim1	CB1	CB1^{Sim1Cre}	Hypothalamus, mediobasal amygdala	Dubreucq et al., 2012
	Transgene	NSE	FAAH	FAAH-NS	CNS	Cravatt et al., 2004
Opioid	Cre/loxP	Nav1.8	Δ (Oprd1)	Oprd$^{NaV1.8Cre}$	Peripheral NaV1.8 primary nociceptive neurons	Gaveriaux-Ruff et al., 2011
CRF	Cre/loxP	CamKII	CRF1	Crfr1^{CamKIICre}	Hippocampus, neocortex, striatum, amygdala	Müller et al., 2003
	Cre/loxP	NEX	CRF1	Crfr1$^{Glu-CKO}$	Cortex, hippocampus	Refojo et al., 2011
	Cre/loxP	Dlx5/6	CRF1	Crfr1$^{GABA-CKO}$	Reticular thalamic nucleus, GP, septum	Refojo et al., 2011
	Cre/loxP	DAT	CRF1	Crfr1$^{DAT-CKO}$	Substantia nigra, VTA	Refojo et al., 2011
	Cre/loxP	Sert	CRF1	Crfr1$^{5-HT-CKO}$	Dorsal and median raphe nuclei	Refojo et al., 2011
	Cre/loxP	CamKII	CRF	CRF-COE	Limbic regions of the forebrain	Silberstein et al., 2009

In summary, these results show the crucial role of NMDA, AMPA receptors, or vGlut2 transporter and their interaction with the dopaminergic reinforcement system in appetitive memory formation and extinction, as well as the modulatory role of mGlu5 receptors in these processes. Similar neurobiological mechanisms might also apply to alcohol-induced memories.

3.2 GABA System

Conditional mutants targeting the α1 or α6 subunits in different neuronal populations are available (Sonner et al. 2005; Saarelainen et al. 2008). In addition, the $GABA_B$ receptor floxed mouse (Haller et al. 2004) represents a potential tool that, in addition to solve the problem of lethality as reported in some cases, allows to induce the deletion in a site-specific manner. Thus, the use of this advanced conditional mutant would provide a unique opportunity to achieve more clear conclusions about the role of GABA in alcohol-related phenotypes.

3.3 Dopamine and Serotonin Systems

Given the importance of both monoamines during the development, genetic deletion of any of the dopaminergic or serotonergic components leads to a very strong phenotype, sometimes lethal. Thus, it could be particularly interesting to achieve conditional mutations on these systems. Unfortunately, there is only one conditional model that has been described so far, where the vesicular monoamine transporter VMAT2 is selectively deleted in the serotonin transporter containing neurons, using a combined Cre/lox system approach (Narboux-Nême et al 2011). In this mouse, there is a major depletion in serotonin, but other monoamines are not affected. However, there are increased 5HT1A receptor levels as an adaptative response to reduced serotonin transmission, which is not surprising, given the important role of serotonin during the development. Though this conditional model represents a much more improved approach to study the role of a particular target in a site-specific way, still, given the importance of serotonin system during the development, only a time-specific (in addition to site-specificity) conditional deletion could provide an adequate model to study the role of serotonin system in alcohol-related behaviors.

3.4 Endocannabinoid System

Conditional mutants for CB1 or FAAH have been already developed. For instance, by the use of a neural-specific FAAH knock-out (Cravatt et al. 2004), the role of central and peripheral fatty aminoacids systems can be dissected. But the most advanced progress has been done targeting the CB1 receptor. Emerging studies with the recently developed conditional mutants for CB1 receptor in forebrain-specific interneurons (Marsicano et al. 2003), GABA-specific (Monory et al. 2006), glutamate-specific (Kleppisch et al. 2003), D1 receptor-specific (Monory et al. 2007) or hypothalamus and mediobasal amygdala-specific (Dubreucq et al. 2011) neurons, have demonstrated how different neuronal subpopulations mediate different effects of THC, synaptic transmission, or plasticity. Therefore, these mouse models represent potent and advanced genetic tools to neuroanatomically,

functionally, and behaviorally dissect the differential involvement of the ECS in alcohol use and addiction.

Furthermore, other components of the ECS have also been targeted. Though not in a site-specific manner, the recently developed mutant for MAGL or DAGL, the endocannabinoid 2-AG inactivating and synthesis enzymes, respectively (Pan et al. 2011; Zhong et al. 2011; Gao et al. 2010; Yoshino et al. 2011), which show alterations in endocannabinoid-mediated signaling and plasticity, represent excellent models to test 2-AG-mediated actions in alcohol addiction.

3.5 Opioid System

A single conditional mutant has been created for the δ-opioid receptor in nociceptive sensory neurons (Gaveriaux-Ruff et al. 2011). This model can be potentially used to dissect central and peripheral effects mediated by alcohol.

3.6 Corticotropin Releasing Factor System

Genetic deletion of the CRF system components results in impaired neuroendocrine and behavioral response to stress, among other deficits, due to the important role of this system in the periphery. These limitations can be circumvented by the use of the very recently developed conditional and site-specific mutant models. For example, conditional mice that overexpress CRF only in limbic-resticted areas (Silberstein et al. 2009), the function of the HPA axis is not significantly altered. However, the more advanced approaches have been achieved with the CRF1 receptor. Extra-hypothalamic CRF1 receptor deletion, by the use of a CNS-specific promoter (Schmidt et al. 2006), already tested for alcohol, or the forebrain-specific deletion, using a Camk2 promoter (Müller et al. 2003) have an intact HPA axis. In addition, the more recently developed CRF1 receptor mutations in GABAergic, dopaminergic, glutamatergic, and serotonergic neuronal populations (Refojo et al. 2011) have elucidated a bidirectional role for CRF1 receptors in glutamatergic and dopaminergic neurons in mediating emotional responses. Therefore, the use of these recently developed advanced CRF genetic models for the study of alcohol-related phenotypes, will improve the somewhat limited knowledge obtained with the global mutants so far, in addition to provide a more clear understanding about the interactions of the stress system and ethanol.

4 Conclusions

The use of genetic models in the study of alcohol-related phenotypes has provided a better understanding of the underlying molecular processes and the genetics involved in the development of excessive alcohol consumption. However, most of

these studies have focused mainly on the initiation phase of alcohol consumption, being other stages less examined or, sometimes, not examined at all (Table 9). Therefore, there is a need for more investigations on how a gene of interest influences the transition from controlled to compulsive alcohol use, by testing phenotypes associated with later stages of the addiction cycle, and its interaction with environmental factors, including excessive drinking models, extinction, reinstatement, or craving, and relapse to alcohol (Table 9). Furthermore, very recently the so-called 3-criteria model, which has initially been introduced to study cocaine addiction in rats (Deroche-Gamonet et al. 2004; Belin et al. 2008; Kasanetz et al. 2010), has now been adapted to study alcohol addiction in mice (Radwanska and Kaczmarek 2011). In this model mice have extended access to alcohol for 70 days, followed by the evaluation of 3 criteria of addiction-like behaviors, including (i) the motivation for alcohol in a progressive-ratio schedule of reinforcement; (ii) persistent and compulsive alcohol seeking and taking during signaled 'no alcohol' periods, and (iii) when subjected to punishment; and finally the intensity of relapse after alcohol withdrawal (ADE). Clearly, further studies are needed to confirm the validity of the 3-criteria model in mice. Nevertheless, the potential use of genetically modified mice in the 3-criteria model may provide great success for defining the molecular components involved in alcohol addition.

One major advantage of genetic interventions compared to pharmacological interventions is the target specificity. Whereas most pharmacological ligands have also unspecific effects at other non-target sides, and it takes often years to discover those unspecific effects, genetic tools usually provide excellent target specificity, i.e. a knock-out is a knock-out. However, there are limitations and shortcomings of the findings obtained with the conventional mutant and transgenic models, that include the majority of the studies presented here (Table 9). Thus, it is known that targeted gene mutations, whether obtained by knock-out technology or transgenic overexpression, leads to compensatory processes, especially during development. This fact raises the uncomfortable, and much avoided question of how much the observed phenotype derives from the gene of interest or unknown neuroadaptive processes. However, the introduction of brain-site specific and inducible mouse models in alcohol research greatly assist to obtain a more accurate understanding of the neurobiological mechanisms underlying alcohol addiction.

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Part III
Clinical Phenotypes and Preclinical Models Thereof

Modeling Alcohol Self-Administration in the Human Laboratory

Ulrich S. Zimmermann, Sean O'Connor and Vijay A. Ramchandani

Abstract This review focuses on 27 studies employing experimental alcohol self-administration (ASA) in humans which were published between 1989 and 2010. Twelve studies enrolling healthy, non-dependent social drinkers (HSD) were aimed at evaluating physiological and behavioral determinants of alcohol-induced reward or modeling situations of increased risk to develop alcohol use disorders. The remaining 15 studies tested the effect of medications such as naltrexone, nalmefene, nicotine, mecamylamine, varenicline, gabapentin, aripiprazole, and rimonabant on ASA. The participants were either HSD or non-treatment-seeking alcoholics (NTSA). In 25 of these studies, the subjects ingested alcohol orally and reached a mean peak blood alcohol concentration (BAC) during baseline conditions between 43 and 47 mg% (0.043–0.047%). Two recent studies employed computer-assisted self-infusion of ethanol (CASE), where subjects press a button to request multiple sequential alcohol exposures intravenously instead of drinking. This method has been demonstrated to be safe and provides increased experimental control of BAC and keeps subjects blind concerning the amount already self-administered. Peak exposures in the CASE studies ranged from 60 to 80 mg% in HSD and up to 240 mg% in NTSA.

Keywords Alcohol · Self-administration · Human · Alcohol dependence

U. S. Zimmermann (✉)
Department of Psychiatry and Psychotherapy, University Hospital,
Technische Universität Dresden, Dresden, Germany
e-mail: Ulrich.Zimmermann@uniklinikum-dresden.de

S. O'Connor
Indiana University School of Medicine, Indianapolis, IN, USA

V. A. Ramchandani
Laboratory of Clinical and Translational Studies,
National Institute on Alcohol Abuse and Alcoholism,
National Institutes of Health, Bethesda, MD, USA

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Contents

1 What Can We Learn From Studies on Experimental Self-Administration of Substances Other Than Alcohol? ... 317
1.1 Route of Self-Administration ... 317
1.2 Pharmacokinetic Determinants of Self-Administration ... 317
1.3 Modeling Different Aspects of Dependent Behavior ... 318
1.4 Testing Medications for Their Potential to Attenuate Substance Use ... 318
1.5 Effect of Alcohol Administration on Self-Administration of Other Addictive Drugs ... 318
2 Ethical Issues in Human Alcohol Self-Administration ... 319
2.1 Alcohol Administration to Alcohol-Dependent Subjects ... 319
2.2 Effect of Participating in Studies Involving Experimental Alcohol Administration on Subsequent Drinking Behavior ... 320
3 Studies Involving Oral Alcohol Self-Administration ... 320
3.1 Early Studies: 1965–1975 ... 320
3.2 General Methods and Design of Recent Alcohol Self-Administration Studies ... 323
3.3 Specific Aims and Results of Oral Alcohol Self-Administration Studies ... 331
4 Studies Using Intravenous Alcohol Self-Administration ... 332
4.1 Why Use Intravenous Rather Than Oral Alcohol Administration? ... 332
4.2 Basic Principles of i.v. Alcohol Administration to Humans ... 335
4.3 Development of Computer-Assisted Self-Infusion of Ethanol (CASE) ... 339
4.4 Results of CASE Studies ... 339
4.5 Directions of Future Developments in Intravenous Alcohol Self-Administration ... 343
References ... 348

Experimental alcohol administration in the laboratory has a longstanding tradition in both animal and human research (see Kalant 1998, for a review). For the most part, alcohol intake was controlled by the experimenters, who sought to administer standardized dosages, which were often scaled to body weight. Outcome variables were various aspects of alcohol's effects; behavior in animals, self-report in humans, or physiological measures in both. In humans, such parameters are complicated; some closely linked to addictive behaviors, but difficult to quantify (e.g. alcohol-induced craving), others easily measured, but with limited relevance to addictive behaviors (e.g. alcohol-induced heart rate changes). These limitations are minimized when alcohol consumption, the behavior of ultimate relevance for alcoholism, is made the dependent variable of laboratory experiments. This concept logically leads to the laboratory model of alcohol self-administration. The unifying theme of self-administration paradigms, as reviewed here, is the feedback loop between the behavior of self-administering and the ensuing perception of pharmacological effects (Fig. 1), which can promote or block further self-administration and characterize an alcohol-specific phenotype.

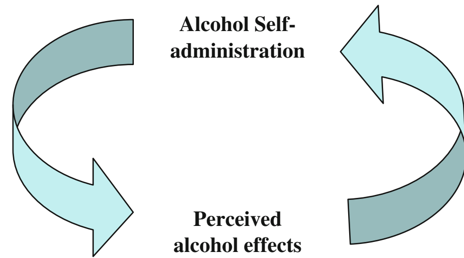

Fig. 1 Closed feedback-loop during alcohol self-administration experiments

1 What Can We Learn From Studies on Experimental Self-Administration of Substances Other Than Alcohol?

Laboratory self-administration studies have focused on heroin, cocaine, cannabis, amphetamines, nicotine, and benzodiazepines (Comer et al. 2008; Haney 2009; Haney and Spealman 2008; Justinova et al. 2005; Koob 2009; Panlilio and Goldberg 2007) more frequently than on alcohol. These studies have defined the essential principles of self-administration research in humans, and have informed studies with alcohol.

1.1 Route of Self-Administration

Researchers have used either the naturalistic route typically exercised by substance users (e.g. smoke, sniff, and ingest), or some refined route which can only be achieved in the laboratory setting (e.g. i.v. injection of controlled dosages). The former more closely mimics the real world, but can confound self-administration driven by pharmacologically induced reward with drug-related cues, especially for substances comprising a mixture of active compounds, such as tobacco (Sofuoglu et al. 2008). For cannabis products, the i.v. route has only been tested in animals (Justinova et al. 2005).

1.2 Pharmacokinetic Determinants of Self-Administration

Several studies demonstrated that during human self-administration of heroin, cocaine, and cannabis, the rewarding effects and preference against placebo rises with dose and rate of delivery (Haney and Spealman 2008; Justinova et al. 2005). This effect could be observed when comparing different routes of administration (e.g. smoking vs. oral) and comparing different infusion rates of i.v. administration.

1.3 Modeling Different Aspects of Dependent Behavior

Self-administration has been used to develop models for the different aspects and stages of substance dependence (Comer et al. 2008; Panlilio and Goldberg 2007). Unconditional free access provides an integral measure of liking, wanting, and tolerating a drug. “Liking” can be specifically assessed using choice paradigms, where the effects of a psychotropic drug and a placebo or comparison drug are blindly sampled by the subject, who thereafter is given the opportunity to chose which of the preparations to self-administer. Drug “wanting” can be dissected from “liking” by operant responding, which involves either fixed or progressive ratios of reinforcement. Another frequently used method to assess “wanting” uses alternative reinforcers. For example, behavioral economics procedures that use money as alternative reinforcer by manipulating the unit price of the drug and measuring consumption across a range of prices to determine elasticity of this behavior. The same goal can be achieved by forcing subjects to choose between self-administering the drug and obtaining an alternative reinforcer.

1.4 Testing Medications for Their Potential to Attenuate Substance Use

Finding medications that help drug-dependent patients reduce their substance intake is a paramount goal in addiction medicine. Testing a medication’s effect on laboratory self-administration can serve as a predictor of its clinical potency, and has been employed for several combinations of drugs of abuse and respective potential medications (Comer et al. 2008; Haney 2009; Haney and Spealman 2008). Medications which effectively reduce heroin use in clinical settings also consistently attenuated heroin self-administration in the lab. Pharmacotherapy to attenuate cocaine dependence has been less successful, but recent promising clinical results with modafinil are paralleled by findings that modafinil also reduces cocaine self-administration in the laboratory. On the other hand, some other drugs neither helped cocaine-dependent patients nor reduced laboratory self-administration, but did reduce subjective cocaine effects (Haney and Spealman 2008). Thus, laboratory self-administration may be a better paradigm to predict a drug’s effect on substance use than measuring subjective effects or craving.

1.5 Effect of Alcohol Administration on Self-Administration of Other Addictive Drugs

Alcohol and other substances of abuse are frequently used together and clinical observation suggests that there is a positive feed-back loop, with exposure to alcohol stimulating the consumption of other drugs and vice versa. This

interrelation has been experimentally verified for the alcohol–nicotine interaction. Studies have showed that nicotine self-administration was increased compared to placebo if subjects were given alcohol prior to the test session (Griffiths et al. 1976; Henningfield et al. 1984; King et al. 2009). Experiments assessing how prior nicotine administration changes alcohol self-administration are reviewed below (see Sect. 3.3).

2 Ethical Issues in Human Alcohol Self-Administration

Experimental administration and self-administration of ethanol in humans invokes an array of potential ethical concerns. In order to help research grant applicants and Institutional Review Boards (IRBs) to weigh risk versus benefits for the multitude of conceivable study protocols and populations, the National Advisory Council on Alcohol Abuse and Alcoholism of the USA agreed on "Recommended Guidelines on Ethyl Alcohol Administration in Human Experimentation", last updated in 2005 (National Advisory Council on Alcohol Abuse and Alcoholism 2005). These comprehensive guidelines discuss issues such as risk–benefit balance, informed consent, subject selection, and confidentiality. Specific reflections are dedicated to the protection of alcohol-naive individuals, the inclusion of populations at risk for dependence or already dependent, taking into account stages of treatment and duration of abstinence in the latter. Other considerations address younger and elderly populations, the use of deception methods, alcohol exposure levels, access to medical backup services, procedures immediately following administration, follow-up, and compensation for participation.

2.1 Alcohol Administration to Alcohol-Dependent Subjects

The ethics of administering alcohol to alcoholics is one of the most controversial issues in addiction research. Clearly, there are important research questions which can only be answered using this approach. In those cases, the NIAAA guidelines recommend inclusion of alcoholics who are not currently seeking treatment. A group of scientists recently published a comment on this subject (Enoch et al. 2009), addressing questions such as whether the results of studies in non-treatment-seeking alcoholics can be extrapolated to treatment-seeking alcoholics, whether alcohol administration studies should recruit alcoholics seeking harm reduction treatments, the risks of a research intervention during the recovery of abstinent treatment-seeking alcoholics, whether newly abstinent alcoholics truly can give informed consent, whether benefits to the society can outweigh the risk to the individual, and what can be done to minimize these risks. The authors conclude that "very little research is currently available to support ethical concerns, and ethical arguments have largely been based on philosophical notions and belief systems", and demand more studies regarding these ethical issues.

2.2 Effect of Participating in Studies Involving Experimental Alcohol Administration on Subsequent Drinking Behavior

There are currently two published empirical studies that examined whether participating in laboratory drinking studies increases the risk of subsequent alcohol consumption in real life (Drobes and Anton 2000; Pratt and Davidson 2005). Alcohol administration was experimenter-controlled in order to achieve a breath alcohol concentration (BrAC) of 80 mg% (i.e., 0.08%). Drinking behavior was assessed using the time line follow-back interview (Sobell et al. 1996) covering the 6 weeks prior to and the same time period following the experiments. Participants were healthy volunteers and non-treatment-seeking alcoholics. The latter were briefly informed about impeding health hazards of their drinking after completion of the experiment. Non-treatment-seeking alcoholics significantly reduced their alcohol intake after participation, while no difference was noted in healthy volunteers.

> As a convention for this chapter, the term "breath alcohol concentration" (BrAC) is used for measurements which are obtained using a breathalyzer, even though the results are converted to units of blood alcohol concentration. The unit used here is mg% of ethanol in whole blood. For example, 80 mg% is the legal driving limit in many states and is equivalent to 0.08% or 0.8‰.

The absence of participation-induced risk does not appear to depend on the route of alcohol administration. Preliminary data in 21 participants of computer-assisted self-infusion of ethanol (CASE) confirm these results in healthy, non-dependent subjects, some with a positive family history of alcoholism (Zimmermann et al. 2009). The mean number of drinks decreased slightly from 46.3 during the 4 weeks preceding the experiments to 37.7 during the 4 weeks after participating in two CASE sessions. During the same interval, the number of drinks per drinking day decreased from 4.7 to 3.5 ($t[20] = 1.95$; $p = 0.066$; t-Test for paired samples, see Fig. 2).

3 Studies Involving Oral Alcohol Self-Administration

3.1 Early Studies: 1965–1975

The modern era of scientific research into the pharmacological actions of ethanol in humans began with the seminal work by Mendelson and La Dou (1964). While this paper described a temporally programmed schedule of drinking rather than self-administration, the first description of operant alcohol self-administration was

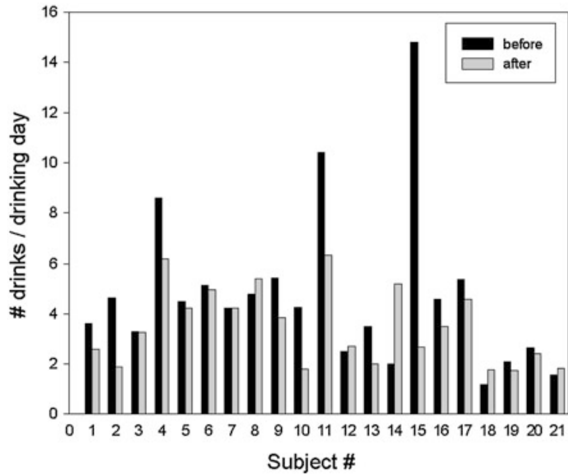

Fig. 2 Drinks per drinking day during the 4 weeks before vs. 4 weeks after participating in 2 CASE sessions in 21 subjects

given by Mello and Mendelson (1965). A series of papers followed, outlining important methodological issues and establishing insights into experimental determinants of alcohol self-administration. This work was reviewed by Mello (1972) and by Bigelow et al. (1975).

3.1.1 General Methods

The methods of these early studies differ in many aspects from those of more recent works. The study population consisted entirely of chronic alcohol-dependent patients, with a typical sample size of 2–5 subjects per study. Given their high urge to drink, no rationale or other specific instructions were given as to why or how they should drink. Most self-administration experiments were performed on research wards and spanned several weeks. Some protocols tested subjects in groups of 2–4 (Bigelow and Liebson 1972; Mello and Mendelson 1965), but later papers warned against the effects of socializing and recommended single testing (Bigelow et al. 1975). Alcohol was available during most hours of almost all days, resulting in maximum BrACs averaging 300–400 mg% (0.3–0.4%) in studies with unrestricted operant self-administration (Mello and Mendelson 1965, 1970). This pattern of high, but unstable consumption led researchers to employ various forms of constraint on self-administration. Successful methods included increasing the workload or reinforcement ratio of operant responding, monetary reinforcement of rejecting drinks, increasing the time interval before the next drink became available, or punishment (e.g. restriction to bedroom, social isolation, and receiving pureed food instead of regular hospital meals) contingent upon consumption exceeding a predefined limit.

3.1.2 Overall Results

Alcohol self-administration decreased when the work required to obtain alcohol increased. For example, increasing the fixed ratio (FR) of lever presses required to get a drink (containing 11 g ethanol) from 100 to 1,000 accomplished little change, but the number of drinks consumed decreased to about 50% at FR 3000 and to about 10% at FR 5000 (Bigelow and Liebson 1972). Alcohol-dependent subjects self-administered more alcohol than non-dependent controls. Offering beverages which were either high or low in alcohol congener content (bourbon versus vodka or laboratory ethanol) did not systematically change self-administration. The use of a priming drink preceding a self-administration experiment showed inconsistent effects in these early studies involving alcohol-dependent participants, but increased drinking when consumption was slightly discouraged by increasing efforts required to obtain alcohol, or applying negative consequences of drinking (Bigelow et al. 1977). The same study also found that the number of consumed drinks increased with their alcohol content (varying between 2 and 14 g) and their concentration (1–47%) if the alcohol content was kept constant.

3.1.3 Comparison with Animal Studies

Operant self-administration is a widely used method in animal research (see Sanchis-Segura and Spanagel (2006), for review) and often involves progressive ratios (PR) of reinforcement in order to determine the break point beyond which an animal ceases to respond for alcohol. Although this method closely resembles the above-described human studies, some differences must be considered before comparing them. First, operant training in rodents begins at a FR 1, and may be gradually increased to FR 4 or 5. Typical baseline break points in PR testing range about 10–15 responses to gain a reinforcement, and can be about doubled by manipulations such as forced alcohol exposure and deprivation (Brown et al. 1998; Ciccocioppo et al. 2003; Gilpin and Koob 2010; Oster et al. 2006). The human studies described above suggest that in alcohol-dependent patients, raising ratios up to FR 1000 left self-administration unchanged. No PR paradigm was performed, nevertheless it can be concluded from the various FR experiments that the break point was around FR 5000, which is about 500 times higher than in rodents. More recent PR studies in healthy social drinkers demonstrated baseline break points about 280, which was reduced to about 125 by acute tyrosine and phenylalanine depletion (Barrett et al. 2008). Another study found break points of about 850 with concomitant cigarette smoking, compared to 600 when smoking denicotinized cigarettes (Barrett et al. 2006). Clearly, one major underlying reason for differences between animals and humans points is human insight into the nature of the experiment and the ability to anticipate the outcome of prolonged sequences of behavior. One consequence is that in human operant response studies, the work for the next reward can take up to 15 min or more, which eventually narrows the

bandwidth of possible ratios. A more comprehensive discussion of this issue is given in the Sect. 4.5.1 at the end of this chapter.

3.2 General Methods and Design of Recent Alcohol Self-Administration Studies

A 15 year-long hiatus followed these initial human alcohol self-administration (ASA) studies. We were unable to identify reports on ASA studies in the late 1970s and 1980s, until the developmental work in 1989 by de Wit et al. (1989), who showed that ASA was related to individual differences in subjective alcohol effects. Since then, we found 25 published studies on oral ASA in humans (see Table 3). Major differences compared to the early studies included the use of larger sample sizes, studying healthy social drinkers, and much shorter duration of the experiments; usually with a maximum of 3 self-administration sessions lasting no more than 3 h each. These studies bear important similarities and differences in their general methods, as discussed below. They can be compared regarding their main outcome criteria during baseline conditions, i.e., placebo treatment or sham intervention in the control group. Primary outcome measures which are reported in most papers were the average amount of alcohol self-administered (grams), the average peak BrAC, and percentage of subjects never using the self-administration. Secondary outcome measures varied between studies and included time to first sip, latency to finish a drink, time of terminating drinking, or the rate of drinking during various phases of the experiment.

3.2.1 Comparison of Methods Used

Subjects

Recent ASA studies have been performed in non-treatment-seeking alcoholics (NTSA, 7 studies) and in healthy social drinkers (HSD, 19 studies) and in heavy, but not alcohol-dependent drinkers (Nesic and Duka 2006; George et al. 2010). Inclusion and exclusion criteria usually define a range of allowed average drinks and drinking days per week and exclude other substance use disorders and substantial adverse health consequences from drinking. A mandatory brief counseling session is usually provided to NTSA after the study is finished. Interestingly, HSD and NTSA do not differ in the self-administration outcomes described in Table 1.

Setting

ASA experiments were conducted in variable settings in an effort to standardize external alcohol-related cues. Some researchers tried to encourage ASA by testing subjects in settings such as a bar-like or living room-like laboratory or even an

Table 1 Effect of categorical methodological variables on self-administration outcomes

Comparison	Average grams alcohol self-administered[a, b]	Average maximum reached BrAC (mg%)[b]	% of participants who did not self-administer at all[b]
Healthy social drinkers vs. non-treatment-seeking alcoholics	36.9 ± 15.7 vs. 34.5 ± 15.4 (17 vs. 7 studies)	45.3 ± 14.9 vs. 44.0 ± 22.6 (7 vs. 2 studies)	22.0 ± 24.0 vs. 26.2 ± 14.2 (10 vs. 5 studies)
Testing subjects singly vs. in groups	35.0 ± 15.4 vs. 39.8 ± 16.3 (18 vs. 6 studies)	44.4 ± 15.8 vs. 47.0 ± 18.4 (7 vs. 2 studies)	25.6 ± 21.4 vs. 8.5 ± 0.7 (13 vs. 2 studies)
Alternative reinforcer absent vs. present	37.2 ± 16.8 vs. 35.7 ± 15.2 (8 vs. 16 studies)	42.5 ± 24.8 vs. 45.7 ± 14.3 (2 vs. 7 studies)	15.5 ± 20.1 vs. 26.3 ± 21.2 (4 vs. 11 studies)

Data are mean ± SD of the indicated number of studies providing the respective data. No significant differences were detected using two-sided T-tests for independent variables

[a] estimated in a hypothetical subject weighing 78 kg

[b] during baseline conditions, i.e., in control subjects treated with placebo or sham intervention

actual bar, while others used standard research lab settings. Eighteen studies investigated subjects singly, while 8 studies tested subjects in groups of 4 or even up to 9 individuals. Contrary to expectations, subjects tested in groups apparently did not consume more than those tested singly (see Table 1).

Reinforcement Contingencies and Alternative Reinforcers

Frequently used reinforcers that were provided as alternatives to alcohol during self-administration experiments included non-alcoholic beverages and money paid for each drink not consumed. They were employed in an effort to control the very high and unstable amounts of drinking that were observed in the earlier studies with alcohol-dependent subjects. Other reasons to use alternative reinforcers were to test the relative reinforcing value of alcohol against a comparator, to ensure that subjects specifically chose alcohol rather than simply seeking any reinforcement, or simply to ensure that thirst was not a motivation to drink alcohol. In some experiments, alcohol and alternative reinforcers were offered using an operant response paradigm, where subjects were required to work or pay before getting access to alcohol. Such paradigms can be modified by employing fixed ratios of reinforcement (e.g. only every 10th pressing a button is reinforced = FR10), or progressive ratios with a low-starting point (e.g. reinforcements after pressing for 10, then 20, then 40, 80, 160 times, respectively, i.e., PR2 starting at 10). This method is accepted as an approach to behaviorally dissect "liking" from "wanting" alcohol (Berridge et al. 2009; Sanchis-Segura and Spanagel 2006). When recent ASA studies with and without alternative reinforcers were compared, no differences in self-administration could be detected, suggesting that either these studies cannot be reliably compared or that alternative reinforcers do not substantially reduce drinking under baseline conditions.

Instruction of Subjects

Informing participants in ASA studies regarding the purpose of the study and instructing them why and how much they are supposed to consume is a crucial element because it necessarily influences the subject's behavior. Therefore it is surprising that the majority of recent papers did not report on this issue. Some probably did not instruct subjects at all, which may be a serious source of experimental variation because then subjects can have different reasons for drinking which would consequently result in different outcomes. Examples of instructions are: "drink as many drinks as you desire or receive money for drinks not consumed" or "you can either press button A or B. You may press either of these buttons or sit and do nothing. You are paid only for attendance and not for button pressing". Some investigators have used deceptive instructions, e.g., feigning the rationale as evaluating the subject's preference for different alcoholic beverages, and instructing subjects to "drink as much or as little as you like, but be sure to sample enough of each beer to give an accurate rating".

Size of Priming Drinks

All but 6 of the more recent studies involved a mandatory alcoholic priming drink preceding self-administration by 10–40 min. Smelling, tasting, and ingesting a drink, and the subsequent pharmacological effect are cues which can be very effective to increase alcohol craving and should be expected to enhance self-administration. This effect was analyzed in the recent ASA studies, assuming a typical subject's weight of 78 kg to compare studies which prescribed priming drinks relative to body weight to those using a fixed size priming drink. The mean and SD amount of alcohol in priming drinks was 15.2 ± 9.2 g (maximum 31, minimum 8) and did not correlate with the amount of alcohol self-administered or the average maximum BrAC under baseline conditions. Larger-sized priming drinks were associated with a higher percentage of subjects who never made use of the chance to self-administer alcohol (Table 2); this finding was largely, but not entirely due to a study by Drobes et al. (2003). These unexpected results suggest that the main effect of priming is not very strong and that other factors differing between these studies may be more important determinants of ASA.

Size of Self-Administration Drinks

The average alcohol content of drinks in the 24 ASA studies was 36.2 ± 15.4 g, assuming a subject's body weight of 78 kg (minimum 3, maximum 57 g). Two studies did not use drinks of a predefined size. As would be expected, the self-administration drink size was marginally related to the amount of alcohol self-administered ($r = 0.4$, $p = 0.052$, Table 2).

Table 2 Effect of metric methodological variables on self-administration outcomes

	Average grams alcohol self-administered[a, b]	Average maximum BrAC (mg%)[b]	% of participants who did not self-administer[b]
Size of priming dose (grams)[a]	−0.2 (24)	−0.1 (9)	0.6* (15)
Size of self-administration dose (grams)[a]	0.4 (22)	0.3 (6)	0.2 (13)
Duration of experiment (minutes)	0.3 (24)	−0.05 (9)	−0.3 (15)

Data are Pearson correlation coefficients. The respective number of studies entered into the correlation analysis is given in parenthesis

* $p < 0.05$

[a] estimated in a hypothetical subject weighing 78 kg

[b] during baseline conditions, i.e., in control subjects treated with placebo or during sham intervention

Duration of Self-Administration Experiments

The more recent studies used self-administration periods of 104 ± 37 min on average, ranging from 15 to 180 min. There was a trend for longer experiments to produce higher amounts of self-administration (Table 2).

3.2.2 Prototypes of Experimental Paradigms

The methods of most recently published ASA studies can be grouped into one of two prototypical experimental paradigms, the de Wit choice against placebo paradigm and the O'Malley choice against money paradigm. Various research groups have used one of these two methods, usually with only marginal adaptations (see Table 3).

De Wit Choice Against Placebo Paradigm

In 1989, de Wit et al. developed a 7-session experiment which was employed in 5 studies (de Wit et al. 1989, 1999, 2003; de Wit and McCracken 1990; Duka et al. 1998). Subjects sampled alcohol and placebo in color-coded cups for the first 4 sessions and then made blinded choices to obtain either alcohol or placebo during the last 3 sessions. The setting was a living room-like lab where subjects were studied in groups of 4 and were offered/ standard drinks containing (for a 78 kg subject) either 8–12 g of diluted laboratory alcohol or placebo. After a priming drink of 8–23 g, access to drinks was free with the constraint that only one drink was available every 10–15 min. The maximum number of available drinks ranged from 5 to 10 and the duration of self-administration ranged correspondingly between 30 and 180 min. The primary outcome measures were the percent of

Table 3 Synopsis of recent alcohol self-administration (ASA) studies

Reference	Subjects/% males	Intervention/comparison/method	Results
Studies investigating the physiology of alcohol-induced reward			
(de Wit et al. 1989)[a]	12 HSD (all m)	None (developmental study) [BS/3]	Higher ASA in subjects perceiving stronger stimulant effects, lower ASA with stronger sedative effects
(Duka et al. 1998)[a]	18 HSD (all m)	Effect of trait characteristics on ASA [BS/3]	Alcohol expectancy of "sociability" correlates positively with ASA. Intensity of positive subjective alcohol effects predict percentage of subjects blindly choosing alcohol over placebo
(Petrakis et al. 2002)[b]	12 NTSA (all m)	Acute tryptophan depletion vs. balanced amino acid administration [WS/2]	Depletion did not change ASA or craving for alcohol
(Spiga et al. 1997)[c]	4 HSD (all m)	Variation of alcohol dose in drinks and reward contingency in operant response paradigm [WS/24]	ASA increased with dose per drink and decreased with FR at low, but not at high doses per drink
(Barrett et al. 2008)[c]	16 HSD (all m)	Attenuation of dopaminergic neurotransmission by acute phenylalanine/tyrosine depletion vs. sham depletion [WS/3]	Less ASA after active depletion, predicted by alcohol-induced heart rate increase, not rescued by concurrent L-DOPA administration
(Weafer and Fillmore 2008)[c]	26 HSD (54% m)	Correlation between alcohol-induced disinhibition (Go/NoGo task) and ASA [BS/1]	Pronounced alcohol-induced disinhibition is associated with high ASA
Studies investigating models of increased risk for alcohol use disorders			
(de Wit and McCracken 1990)[a]	22 HSD (all m)	Comparison of FHP with FHN subjects [BS/2]	No effect of family history of alcoholism on ASA or on % of sessions where alcohol was chosen over placebo
(de Wit et al. 2003)[a]	37 HSD (65% m)	Exposure to acute social stress vs. no stress [mixed BS and WS/4]	Stress nonspecifically increased self-administration of both alcohol and orange juice
(Roehrs et al. 1999)[c]	20 HSD (60% m)	Subjects with vs. without chronic insomnia [BS/3]	Insomniacs chose ethanol more often and self-administered more alcohol than controls

(continued)

Table 3 (continued)

Reference	Subjects/% males	Intervention/comparison/method	Results
(Nesic and Duka 2006)[c]	32 NDHD (50% m)	Exposure to acute social stress vs. no stress [BS/1]	No main stress effect on ASA, but more ASA in males than females after stress. Males had greater initial rate of consumption than females
Studies investigating the effect of opiate antagonism on ASA			
(Davidson et al. 1996)[c]	16 HSD (63% m)	Naltrexone (NTX) 50 mg/d for 8 days vs. PLA vs. no drug [WS/3]	NTX increased time to 1st sip and reduced mean BrAC. No difference of light vs. heavy drinkers in ASA
(Davidson et al. 1999)[c]	51 NDHD (73% m)	NTX 50 mg/d for 1 week vs. PLA, 2 weeks washout [WS/2]	NTX reduced time to 1st sip, latency to finish a drink, and number of drinks consumed
(de Wit et al. 1999)[a]	24 HSD (71% m)	NTX 50 mg single dose [WS/4]	NTX equally reduced alcohol and placebo self-administration and did not alter % of sessions where alcohol was chosen over placebo
(O'Malley et al. 2002)[b]	18 NTSA (72% m)	NTX 50 mg/d for 6 days vs. PLA [BS/1]	NTX slowed drinking speed and decreased number of drinks consumed and BrAC
(Drobes et al. 2003)[b]	125 NTSA (82% m) and 90 HSD (77% m)	Dose escalation over 8 days of NTX (up to 50 mg/d) or nalmefene (up to 40 mg/d) vs. PLA [BS/1]	Both medications reduced ASA in both populations. NTSA self-administered more than HSD
(Anton et al. 2004)[b]	40 NTSA (73% m)	NTX escalation over 7 days up to 50 mg/d vs. PLA. Immediate vs. delayed availability of ASA after alcohol priming drink [BS/1]	NTX slowed drinking speed and decreased number of drinks consumed, but only if access was delayed by 40 min after the alcohol priming dose. No NTX effect with immediate ASA access
(Krishnan-Sarin et al. 2007)[b]	92 NTSA (73% m)	NTX 50 or 100 mg/d over 6 days vs. PLA in FHP vs. FHN subjects [BS/1]	No main effect of NTX. 100 mg NTX decreased number of drinks consumed by FHP but not FHN. NTX decreased BrAC in FHP, but increased it in FHN subjects
Studies investigating the effect of manipulations of the nicotinergic system on ASA			
(Young et al. 2005)[c]	24 HSD (50% m)	Mecamylamine single dose 7.5 or 15 mg vs. PLA (WS/3]	No main effect of mecamylamine on ASA, but less ASA in the subjects who experienced marked stimulation by alcohol. All subjects smoking < 5 cigarettes/week

(continued)

Table 3 (continued)

Reference	Subjects/% males	Intervention/comparison/method	Results
(Acheson et al. 2006)[c]	34 HSD (64 m)	Nicotine single patch 7 or 14 mg vs. PLA in light smokers [WS/3]	Nicotine increased ASA in males and decreased it in females
(Barrett et al. 2006)[c]	15 HSD (100% m)	Smoking nicotine-containing vs. de-nicotinized cigarettes before and during ASA in light non-dependent smokers [WS/2]	With nicotine-containing cigarettes higher break point to work for alcohol, higher preference for alcohol vs. water, quicker consumption of cigarettes, compared to de-nicotinized cigarettes
(McKee et al. 2008)[b]	20 NDHD (55% m)	Short-term forced abstinence from smoking; single nicotine replacement patch (21 mg) vs. PLA [WS/2]	Nicotine replacement slightly decreased ASA during cigarette withdrawal in daily smokers
(McKee et al. 2009)[b]	20 HSD (80% m)	Varenicline escalating dose over 6 days up to 2 mg/d vs. PLA [BS/1]	Varenicline decreased number of drinks consumed, BrAC, alcohol craving, and subjective alcohol effects in daily smokers
Studies investigating other pharmacologic manipulations of ASA			
(Myrick et al. 2007)[b]	35 NTSA (94% m)	Gabapentin escalation over 7 days up to 1200 mg/d vs. PLA [BS/1]	No gabapentin effect on ASA compared to PLA.
(Voronin et al. 2008)[b]	30 NTSA (83% m)	Aripiprazole escalation over 7 days up to 15 mg/d vs. PLA [BS/1]	Trend for less number of drinks consumed after aripiprazole compared to PLA.
(George et al. 2010)[b]	49 NTSA (80% m)	Rimonabant 20 mg/d over 14 days vs. PLA [BS/1]	No rimonabant effect on ASA. Positive correlation between priming response and ASA across groups

HSD healthy social drinkers, *NTSA* non-treatment-seeking alcoholics, *NDHD* non-dependent heavy drinkers, *FHP* family history positive for alcoholism, *FHN* family history negative, *m* male, *ASA* alcohol self-administration, *NTX* naltrexone, *PLA* placebo, *BrAC* breath alcohol concentration. *[BS/1]* between-subjects design with 1 session, *[WS/2 or 3]* within-subjects design with 2 or 3 ASA sessions

[a] de Wit prototype

[b] O'Malley prototype

[c] other design

sessions where subjects chose alcohol over placebo, and the number of drinks consumed, but not BrAC. This paradigm was exclusively used in healthy social drinkers (HSD) to investigate physiology of ASA in two studies, to model risk situations for heavy drinking in two studies, and to study the effect of naltrexone in one study (Table 3). The total amount of consumed alcohol per session was rather low, averaging 38 g for a subject of 78 kg body weight.

O'Malley Choice Against Money Paradigm

This design first published by O'Malley et al. and later adapted by Anton et al., is the more widely used paradigm by other research groups (Anton et al. 2004; Drobes et al. 2003; George et al. 2010; Krishnan-Sarin et al. 2007; McKee et al. 2008; McKee et al. 2009; Myrick et al. 2007; O'Malley et al. 2002; Petrakis et al. 2002; Voronin et al. 2008). It was most often used to study NTSA but also in 2 studies with HSD (Drobes et al. 2003; McKee et al. 2008) and typically involves one single session, although there are 2 reports using it for a within-subjects comparison (McKee et al. 2008; Petrakis et al. 2002). All but one of these studies aimed at investigating the effect of pharmacological interventions on ASA. The setting was either a standard or a bar-like laboratory where subjects were tested singly. They were offered standard drinks consisting sometimes of standard beverages accommodating the subject's choice of brands, but most often of liquors chosen by the subject which were then diluted to a concentration of 10% (v/v). The size of self-administration drinks was adapted to body weight and for a 78-kg subject contained either 8 or 12 g of ethanol. After a weight-adjusted priming drink (either 16, 23, or 31 g for a 78-kg individual), subjects were presented with a tray of 4 drinks and instructed to "drink as many drinks as you desire or receive dollar amount for drinks not consumed". Not consuming drinks was reinforced by paying the $3 per drink not consumed on the next morning, in addition to the $150 compensation for participating in the ASA experiment. Subjects had one hour to drink, then the remaining drinks were removed and a new tray of 4 drinks was presented with the same instruction. Primary outcome parameters were the number of drinks consumed. Some studies also analyzed BrAC converted to units of blood alcohol concentration and reported the percentage of subjects never making use of the self-administration. In the 7 studies testing NTSA, the mean total alcohol intake per session was 34.5 g (in a 78-kg subject), compared to 35.3 g in the 4 studies involving nondependent, non-treatment-seeking heavy drinkers.

Again, the total amount of alcohol appears surprisingly low in both NTSA and HSD. Possible reasons include that during oral ingestion experiments subjects remember how much they already ingested and, from the taste, they can guess how strong the drinks were. As subjects know that their drinking behavior is being observed by the experimenter, they might be embarrassed to have a lot of drinks out of concern for what the laboratory staff might think of them. This concern might especially apply to nontreatment- seeking alcoholics, who very likely have experienced criticism for their drinking in the past. A second issue possibly impeding alcohol self-administration by ingestion is that the subjects are offered a

fixed number of prepared drinks per hour. The limited number of offered drinks necessarily communicates some expectancy to the subjects as to how many drinks the experimenters think they might or should ingest at the most. Some subjects might be reluctant to take all the possible drinks because they perceive this would be an extreme behavior and they would score top in consumption.

A major difference between these two paradigms is that the O'Malley design includes an alternative reinforcer, i.e., money for not consuming a drink, which is not present in the de Wit design. Since the average consumption in HSD is comparable between the two paradigms (35.3 with alternative reinforcer vs. 38.0 g without), this appears to have only a small impact on laboratory self-administration.

3.3 Specific Aims and Results of Oral Alcohol Self-Administration Studies

A synopsis of the more recent ASA studies is given in Table 3. They can be grouped into three categories according to their aims as follows:

3.3.1 Studies Evaluating Physiology of Alcohol-Induced Reward

Of these 6 studies, 3 were correlational and investigated the relation between specific trait variables or individual differences in alcohol effects with ASA. Three others used different interventions to experimentally manipulate ASA. All but one could substantiate their starting hypothesis.

3.3.2 Studies Investigating Models of Increased Risk for Alcohol Use Disorders

Two of these 4 studies tested the hypothesis that non-alcohol-dependent subjects with risk factors to develop alcoholism self-administer more alcohol compared to low-risk controls. Two other studies used a standardized psychosocial stress task since stress is associated with increased risk for heavy drinking or relapse. Only one of them could fully and another could partly substantiate the main hypothesis.

3.3.3 Studies Applying Pharmacological Manipulations of ASA

There are 7 studies testing the hypothesis that *opiate antagonism* decreases ASA, mostly by administering naltrexone for a week before testing. Four of them used the O'Malley paradigm. In 4 studies, naltrexone unambiguously decreased ASA in both HSD and NTSA. In two other studies this was only true under certain conditions or in subgroups of the study population, while one study in HSD found no specific effect on ASA.

Five other studies applied agonistic or antagonistic manipulations of the *nicotinergic system*, testing the assumption that nicotinergic neurotransmission is

involved in mediating alcohol's rewarding effect and thus enhances ASA. Subjects were HSD and either cigarette chippers or daily smokers. The results of two studies supported this concept, while others found strong modulating effects of sex, tobacco dependence/withdrawal, and alcohol-induced subjective stimulation on ASA. Effects of gabapentin, rimonabant, and aripiprazole on ASA were undetectable or ambiguous.

Overall, the effects of these medications on ASA are reasonably consistent with their utility in treating alcohol dependence, or their effect in daily life, respectively. For example, naltrexone and nalmefene reduce both laboratory ASA and drinking in alcoholics, while rimonabant as well as gabapentin were ineffective in ASA as well as in clinical trials, and aripiprazole showed ambiguous effects in both. On the other hand, nicotine and cigarettes stimulated laboratory ASA which is consistent with the >80% comorbidity between nicotine and alcohol dependence in alcoholics (Kalman et al. 2005) and with the experience of irregularly smoking social drinkers who tend to drink more when smoking. Whether or not varenicline's attenuating effect on ASA translates into clinical efficiency in alcoholics is currently under investigation in two randomized clinical trials. There are currently no reports about medications which do not alter ASA but effectively modify drinking in alcoholics, or vice versa. Therefore, testing a drug's effects on laboratory ASA appears to be a valuable and valid tool to predict its clinical efficacy, paralleling the above-described findings with heroin self-administration (see Sect. 1).

4 Studies Using Intravenous Alcohol Self-Administration

4.1 Why Use Intravenous Rather Than Oral Alcohol Administration?

Ingestion of alcohol leads to substantial variability across subjects in the systemic exposure to alcohol, including that of the brain. The time course of an individual's alcohol exposure depends on the kinetics of absorption, distribution, and elimination. Absorption kinetics are the primary source of variability, subject to the individual's recent history of food and pharmaceutical intake, alcohol concentration of the ingested beverage, rate of drinking, time of day, and gastric emptying. Distribution kinetics vary with gender, age, cardiovascular function, and body mass index. Elimination kinetics depend on BrAC level, first-pass metabolism, genetic polymorphism of the alcohol metabolizing enzymes (alcohol dehydrogenase and acetaldehyde dehydrogenase), and hepatic function. Some of these factors are controllable through experimental design, but the interplay between them yields a significant degree of variability in the time course of the brain's exposure even after attempting to control these factors. Figure 3 depicts results of a laboratory experiment where 44 healthy young adult social drinkers ingested 20% ethanol by volume in fruit juice over 10 min, with the amount of alcohol administered equaling 1.0 g ethanol per liter of the individual's total body water. The experiment was

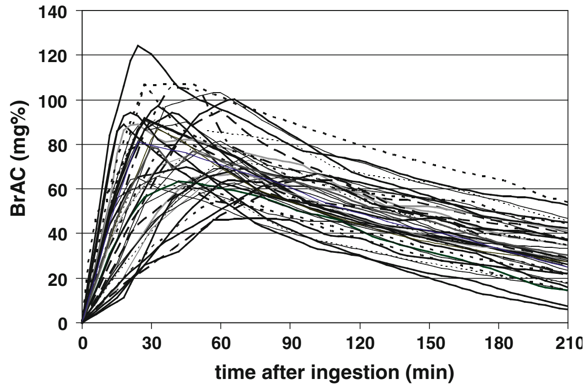

Fig. 3 High variability of individual breath alcohol concentration (BrAC) over time after oral alcohol intake in 44 healthy social drinkers (Reproduced from (Ramchandani et al. 2009) with permission)

conducted in the same setting, at the same time of day, following an identical light meal 2 h before alcohol consumption, and using identical post-ingestion procedures. Interpolation of frequent breath alcohol concentration (BrAC) measurements over the ensuing 3.5 h revealed poor control of the time course of alcohol exposure associated with the oral route of administration (Ramchandani et al. 2006, 2009).

Intravenous infusion of ethanol completely avoids the primary source of variability, and allows for compensation of differences in distribution and elimination kinetics by the use of one or both of two methods of choosing the infusion pump rate. One method is feedback control; varying the infusion rate based on the differences between the desired and measured BrAC over time. The other is calculation of the infusion rate profile using a physiologically based pharmacokinetic (PBPK) model of alcohol distribution and elimination with model parameters tailored to the individual subject. Figure 4 shows steady BrACs over prolonged periods of time, achieved by combining both methods. Fifty healthy young adult social drinkers were infused with 6% ethanol in normal saline to produce a BrAC of 60 mg%, using a pre-calculated infusion rate profile based on a PBPK model of alcohol distribution and elimination, with manual variation of the infusion rates based on BrAC measurements obtained during the experiment.

4.1.1 Advantages of the i.v. vs. Oral Route for Alcohol Administration Experiments

Full Control Over BrAC

When precise adherence to a desired time course of brain exposure to alcohol is desired, the advantage of employing an i.v. route of administration is evident in the preceding figures. Another advantage is experimental flexibility; within a wide range, the prescribed time course of the brain exposure is up to the investigator. Linear or nonlinear ascents, continuous or intermittent intervals of constant exposure, and controlled descent rates can be achieved. With proven PBPK

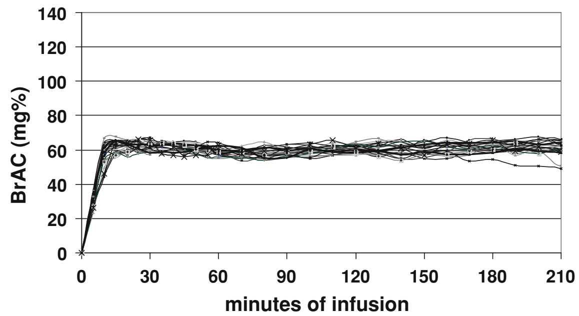

Fig. 4 Low variability of individual breath alcohol concentration (BrAC) over time using i.v. alcohol infusion in 50 healthy social drinkers

models, pre-calculated infusion rate profiles can be used in imaging environments where the risk of motion artifacts and high-magnetic fields preclude obtaining BrAC readings.

Safety

Intravenous infusion enjoys a subject safety advantage over ingestion. Under any circumstances, the BrAC begins to descend immediately and monotonically whenever the infusion is terminated. With oral administration, a reservoir of recently ingested alcohol often remains, and absorption can continue to increase the BrAC long after the administration is terminated.

Improving Experimental Control Using Feedback of BrAC Readings

Offline, accurate PBPK-based estimates of the individual subject's continuous brain exposure can be obtained by fitting the model's output to measured samples of BrAC, given the known infusion rate profile. This capability is limited with ingested alcohol because pharmacokinetic models of alcohol absorption are not reliable.

Blinding Administration and Avoiding Alcohol-Specific Cues

Using the intravenous route, alcohol can be administered without the subject's awareness. Thus, infusion also provides a reliable method for dissociating the response to alcohol administration from demand characteristics such as taste, smell, and familiarity/preference of the source of the alcohol. Conversely, in experiments where the expectations of the response to alcohol depend on the experience of ingesting a beverage, intravenous infusion of alcohol may not be desirable, unless deceiving the subject is an integral part of the design.

4.1.2 Disadvantages of the i.v. vs. Oral Route for Alcohol Administration Experiments

Need for i.v. Line

Successful intravenous infusion of alcohol depends on access to an antecubital vein because blood flow rates in hand or forearm veins are insufficient to dilute the alcohol at the infusion site to concentrations that avoid endothelial irritation. About 5 percent of otherwise well-qualified subjects do not have such access. A similar fraction of other subjects simply do not tolerate catheter insertion without unacceptable anxiety, but both limitations can be determined before scheduling testing.

Medical Skills

Intravenous infusion requires sterile technique in the preparation of infusate and vein catheterization and on-call access to a physician to assure subject's safety and satisfy IRB concerns. The method also requires availability of a medical grade infusion pump; two if a rapid ascent to a target BrAC is desired. Such pumps are expensive, and only a few provide the information required to write drivers for computer-control of the infusion, which is critical for the use of the software for CASE (Kenny et al. 1986).

Mobility of the Subject

Mobility of the subject is somewhat restricted due to the i.v. setup with pumps and the computer. This restriction can be largely avoided, however, if the mounting of the antecubital catheter is secure. The length of the tubing between the infusion pumps and insertion site can be up to 10 m. Thus, experiments can be conducted in settings such as scanners, simulated bars, and driving simulators (or even in cars using battery powered apparatus) if care is taken not to trip over or occlude the tubing.

4.2 Basic Principles of i.v. Alcohol Administration to Humans

4.2.1 Special Pharmacokinetic Features of i.v. Compared to Oral Alcohol Administration

Intravenous administration of alcohol can account for differences in vascular distribution. Alcohol infused into a forearm causes high concentrations of local venous blood alcohol concentrations, but these are diluted with venous blood from

other sources returning to the right cardiac ventricle. From there, alcohol passes the lungs and enters arterial circulation without further relevant concentration changes, since only less than 1% of alcohol is eliminated by pulmonary gas exchange. Transit from insertion site to the left heart occurs in seconds, so venous infusion essentially controls the arterial blood concentration (aBAC). Ethanol diffuses from capillary blood into the tissue, which is initially devoid of any alcohol, following the high-concentration gradient. Thus, the overall venous blood returning from the peripheral tissue has a lower alcohol concentration (vBAC) compared to aBAC during the loading phase of alcohol administration where blood and tissue concentrations are not equilibrated. If alcohol is administered orally, the ascending phase of alcohol concentrations is a combination of absorption and distribution, and similar phenomena pertain. Sustained rapid i.v. administration can progressively increase the gap between aBAC and vBAC and one can exploit the gap in order to control the descending rate of BrAC until equilibration occurs.

4.2.2 Measurement of Brain Alcohol Exposure During i.v. Alcohol Administration

Forensic scientists measured aBAC, vBAC, and BrAC during experimental oral and intravenous alcohol administration. These studies established that BrAC is always closely related to aBAC, but gives a much less accurate measure of vBAC during both oral and i.v. alcohol administration (Jones et al. 1997; Lindberg et al. 2007; Martin et al. 1984) in the loading/absorption phase. Therefore, BrAC can be used to determine aBAC and thus brain alcohol exposure, since the brain tissue equilibrates quickly with aBAC due to its high-flow rate and low-water volume. The consequence for alcohol self-administration studies is that using a breathalyzer provides a quick and accurate estimate of current brain alcohol exposure.

> The best representation of brain alcohol exposure during alcohol administration studies is breath alcohol concentration (BrAC) converted to units of blood alcohol concentration. Venous blood sampling is inappropriate for this purpose.

4.2.3 Simple i.v. Administration Paradigms Using Pre-Calculated Infusion Profiles and Manual Feed-Back of BrAC

Intravenous infusion of alcohol in humans can be accomplished at several levels of the trade-off between simplicity of methods used and performance. Generally, briefer, lower, and more constant exposure concentrations require less complexity in order to achieve satisfactory results, compared to longer, greater, and more variable time courses of BrAC.

Consider a task simply to raise the subject's BrAC to 40 mg% in 5 min without the intent of controlling the subsequent exposure. The simplest approach is to use a pre-calculated fixed infusion rate for 5 min; results will be quite satisfactory if the correct infusion rate is employed. The next level of complexity in performing the same task would be to serially measure BrAC as the fixed rate is infused using the feedback to terminate the infusion at the target BrAC, achieving more accurate BrACs but slightly variable ascent times. Such an approach has been used by Ray et al. (Ray et al. 2006, 2007a, 2007b, 2008; Ray and Hutchison 2004, 2007) in studies investigating alcohol-induced heart rate increase and subjective effects, and the effect of naltrexone and OPRM1 polymorphisms thereupon.

If the intent of the experiment is to first achieve the BrAC target and then to hold it at that value ("alcohol clamping") the trade-off changes. In order to maintain BrAC constant while the intracellular water compartment equilibrates, an exponentially decreasing infusion rate is required, with an asymptote that is equal to the individual's steady-state alcohol elimination rate. The pace of that exponential decrease and the steady-state rate are difficult to pre-calculate without a kinetic model of alcohol distribution and elimination. Van Gerven et al. achieved this task (Zoethout et al. 2009) and used it in a series of studies testing interactions between alcohol and MDMA (Dumont et al. 2008, 2010a, 2010b). They also developed a pharmacokinetic model which was, however, not helpful to improve their method (Zoethout et al. 2008).

4.2.4 Advanced i.v. Administration Paradigms Using A Physiologically-Based Pharmacokinetic (PBPK) Model

Contrary to the above-referenced experiments, our group developed a "physiologically-based pharmacokinetic (PBPK) model of alcohol distribution and elimination" to perform clamping studies, which helped to control BrAC over time periods up to 4 h (Han et al. 2006; Plawecki et al. 2004, 2007, 2008; Ramchandani et al. 1999a). The model was used for studies on the pharmacokinetics of alcohol distribution and elimination, including sex and genetic effects thereupon, and to study genetic influences on alcohol effects and on acute tolerance to alcohol (Blekher et al. 2002; Kwo et al. 1998; Morzorati et al. 2002a; Neumark et al. 2004; O'Connor et al. 1998, 1999; Ramchandani et al. 1999b, 2002, 2006, 2010). It was also successfully used to mimic the time course of BrAC following oral alcohol administration (Ramchandani et al. 2009).

Achieving a *linear* ascent to a higher BrAC target (e.g. 80 mg%) is another situation where a pharmacokinetic model is helpful. Constant infusion rates cannot achieve constant rates of change of BrAC until equilibration in all body water compartments is achieved (requiring ~90 min). Pre-calculating the variable infusion rate profile to achieve linear ascents (or descents) is much easier using a kinetic model. Further, not using feedback when high or long exposures are employed, e.g. in scanner environments, absolutely requires an accurate kinetic

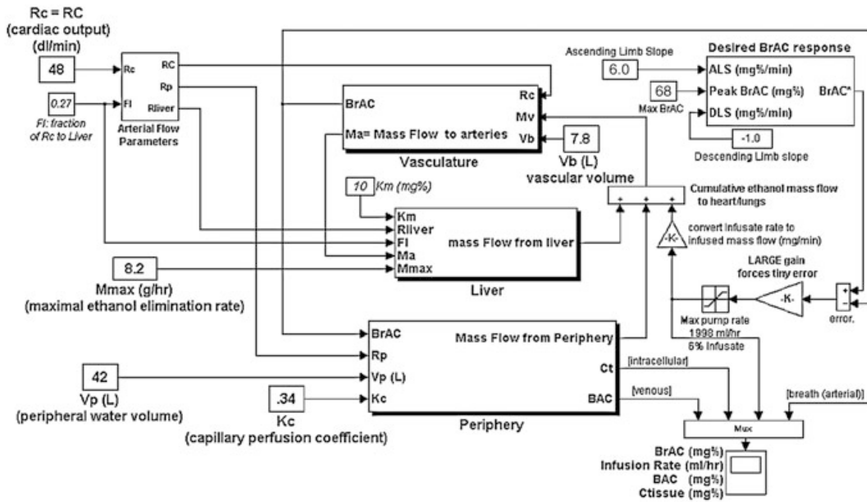

Fig. 5 MatLab Simulink™ physiologically based pharmacokinetic model of alcohol distribution and elimination. See text for detailed explanation

model for safety, unless an extra, preceding session on the same individual documents a successful infusion profile that can be replicated in the scanner.

PBPK models are particularly appealing because they can be tailored to a wide range of individual subjects and even across species (Morzorati et al. 2002b). Ladder models are also appealing because they can comprise as few as three compartments (vasculature, liver, and peripheral body water) for parsimony, or can be expanded to include other compartments of interest (e.g. brain, gut, muscle, and fat). One example of such a model is shown in Fig. 5 above. The user specifies the value of 5 physiological parameters (Rc, Mmax, Vp, Vb and Kc) that tailor the invariant architecture of the 3-compartment ladder model to an individual subject (fl and Km are held constant in humans) The compartments employ differential equations to track the mass flow of ethanol over time. The upper right block labeled “Desired BrAC response” computes the time course of the BrAC that the experiment seeks to achieve. The difference between the output of that block and the modeled BrAC is multiplied by a high gain and fed into the venous vasculature as the infusate rate (assuming a 6% (v/v) ethanol infusate concentration). The solution to the differential equations computes the infusion rate profile that keeps the modeled BrAC very close to the desired time course of BrAC.

The model shown in Fig. 5 was configured to achieve a linear ascent of BrAC at the rate of 6.0 mg%/min until the BrAC reaches 68 mg%, then switch abruptly to a linear descent of−1.0 mg%/min (approximately four times the natural alcohol disappearance rate in humans) for 20 min. Such a time course with contrarious slopes was useful in studying the possibility that the rate of change of brain exposure to alcohol affects the subject’s perceptions of alcohol’s effects. The infusion profile required to achieve this time-BrAC profile results is not intuitive,

requires frequent adjustments of the infusion rate and would be difficult to achieve by operating the infusion pump manually, even if feedback measurements were obtained.

4.3 Development of Computer-Assisted Self-Infusion of Ethanol (CASE)

The technology required to implement the "slopes" experiment described in the preceding paragraph includes a method for solving the differential equations associated with the PBPK model in Fig. 5 in real-time, and driver software for automatic control of the infusion pumps employed. The equations can be solved for the entire duration of the experimental session in a second, so that accurate predictions of the future BrAC are always available. When that technology was included in a set of software that accepted a button push by the subject to initiate an incremental "slopelet" (3.0 mg% per min ascent, increasing the BrAC by 7.5 mg% above the concentration when the button was pushed, then maintaining a–1.0 mg% per minute descent until the next button push or equilibration occurred), one application of the Computer-Assisted Self-infusion of Ethanol (CASE) system became operational (Fig. 6). With CASE, two important improvements over oral self-administration were achieved. The time course of incremental brain exposure is identical in every subject each time the "get more alcohol" button is pushed, and–since the consequence of button-pushing is constantly monitored–overall exposures can be limited to a preset safety level by disabling the button whenever the consequent increase would exceed the limit. Neither capability can be realized in oral experiments.

In CASE experiments, subjects press a button to request a temporary linear increase of their BrAC, which follows a predefined slope (i.e., a "*slopelet*", e.g. linear increase by 7.5 mg% within 2.5 min, followed by a linear descent by–1 mg%/min until the subject's next request, or until the experiment is over. When explaining the CASE setup to the study participants, we call these slopelets "*drinks*" to express the homology and to keep the language easy to understand. Nevertheless, subjects never drink during these experiments, and all alcohol is infused intravenously.

4.4 Results of CASE Studies

While CASE is a very versatile method and can accommodate different experimental paradigms, most studies conducted to date use a paradigm where alcohol was freely available without any work, payment or other preconditions and which

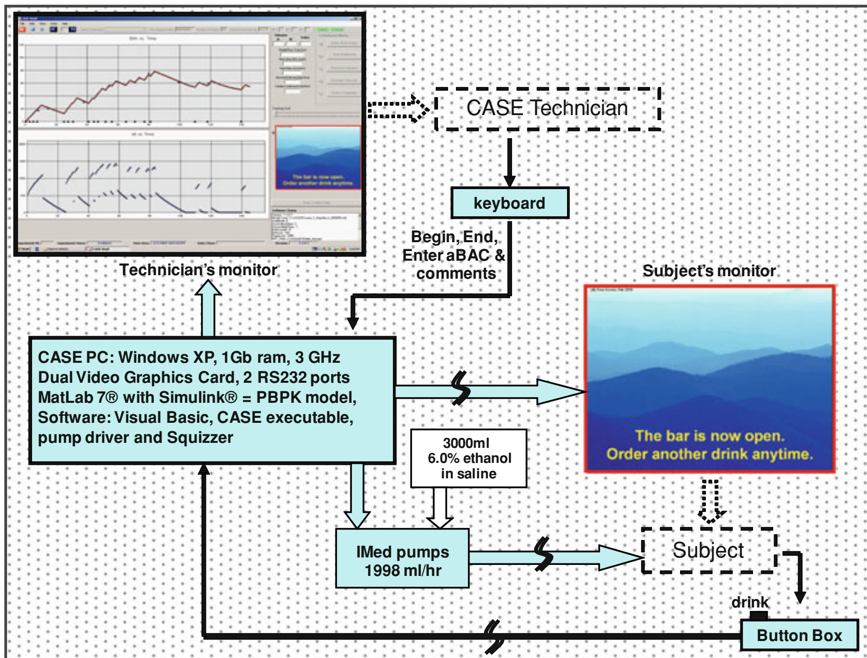

Fig. 6 The Computer-Assisted Self-infusion of Ethanol (CASE) system for alcohol self-administration studies. The technician's screen displays the time course of BrAC, and the infusion rate used to achieve it, for the entire experiment throughout the session. The subject is unaware of these data, deciding to push the "drink" button whenever she/he prefers while the bar is open

therefore was called "Freibier" (German for "free beers"). This is deliberately a very simple paradigm thought to be best suited to test feasibility of i.v. self-administration. Freibier represents the most basic form of an operant response paradigm, where subjects can request increments of their BrAC at any time by pressing a button, provided their BrAC would not exceed a preset safety limit (ranging from 100 to 200 mg% depending on study population and IRB regulations). Self-administration is preceded by a priming interval in which 4 mandatory successive slopelets are administered, raising BrAC to 30 ± 2 mg% in 10 ± 1 min, followed by a waiting interval of another 15 min while BrAC falls to 15.0 ± 2 mg%. Then subjects are instructed to continue requesting alcohol *ad lib*, in order to achieve the enjoyable alcohol effects as they would usually experience when drinking at a week-end party where the drinks were free. Whenever the subject pushes the button, the CASE software calculates the necessary infusion rate and controls the infusion pump to deliver this profile intravenously. During each linear incremental ascension (slopelet), the "request alcohol" button is inactivated to prevent subjects from requesting more alcohol before feeling the full effect of the last request. Outcome measures are the mean and maximum BrAC

achieved during the 2.0 h of voluntary self-administration that follows the 25 min priming/waiting interval, and the number of alcohol requests in that interval. Two published and one yet unpublished study produced the results described below.

4.4.1 Development of the Freibier Paradigm and Influence of the Rate of BrAC Change

The first CASE/Freibier study tested practicability and reliability of the paradigm (Zimmermann et al. 2008). Nine healthy social drinkers participated in 3 sessions each. The increment of BrAC always was 7.5 mg%, but the latency between pushing the button and reaching the new BrAC peak was varied, being 2.5 min on the first day and being randomly changed to 1.5 or 3.5 min on days 2 and 3 in a crossover design in order to find out whether subjects preferred the slower or the quicker rate of change. For an example of these experiments in one individual see Fig. 9. Maximum observed BrAC was 76.5 ± 26.3 mg% on average across all experiments. When grouping days 2 and 3 according to incremental BrAC peak latency (1.5 vs. 3.5 min), peak BrAC achieved and the number of requests in the session were significantly higher with the faster rise and all three outcome measures were significantly correlated between days, demonstrating good test–retest stability. No such correlations were found between the first and either of the following days, suggesting an advantage for conducting a familiarization session in subsequent studies. These results are consistent with the general concept that drugs of abuse are more rewarding, more addictive, and are consumed in higher amounts if their route of administration warrants quicker access to the brain. By providing control over the kinetics of BrAC change, CASE is the first method allowing study of this aspect with regard to alcohol. The conclusion was that the CASE/Freibier paradigm provides a practical and safe method for alcohol self-administration, resulting in considerable alcohol exposure. The time course of BrAC was reproducible and corresponded to subjective ratings of craving and alcohol effects.

4.4.2 Sensitivity of CASE to Family History for Alcoholism

In the second study, 22 healthy non-dependent social drinkers, (aged 21–22 years) comprised a sample with a differential familial history of alcoholism (Zimmermann et al. 2009). 12 FHP subjects (4 females) had an alcoholic first-degree relative; 10 FHN (3 females) had none. All subjects completed the 2-session study with the familiarization and subsequent groups-comparison session separated by at least one week. All but 1 subject made considerable use of the opportunity to self-administer alcohol. All 3 outcome measures were significantly interrelated between days. Maximum BrAC for FHN vs. FHP participants was 61.3 ± 40.1 vs. 85.1 ± 33.3 mg% on the first day ($t\ (20) = 1.1$ n.s.) and 50.5 ± 22.7 vs. 81.6 ± 29.2 on the second ($t(20) = 2.8$, $p < 0.05$), again demonstrating the

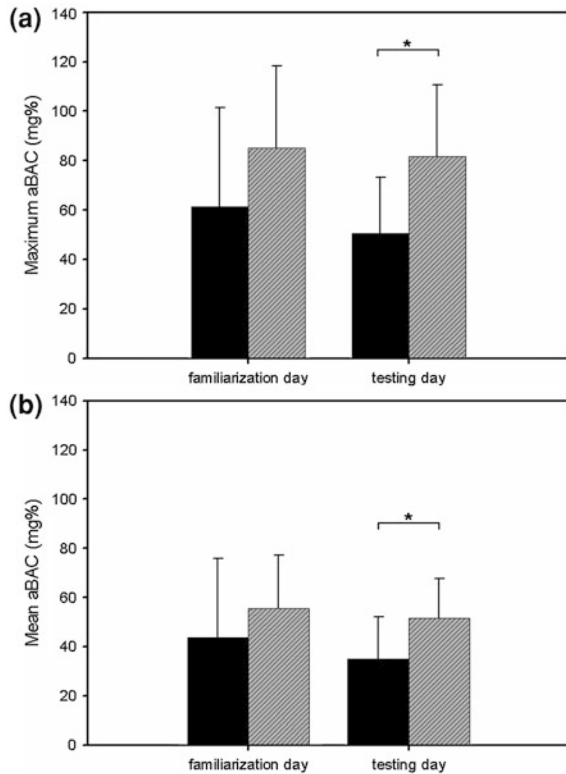

Fig. 7 a, b Mean and maximum arterial blood alcohol concentration (aBAC) throughout the self-administration period of two consecutive CASE experiments. First day = familiarization session; second day = testing session. Black bars: Family history negative controls; hatched bars: family history positive high-risk subjects. Error bars are standard deviation. Reproduced from (Zimmermann et al. 2009) with permission

advantage of a familiarization session (Fig. 7). Mean BrAC and the number of drinks requested were also significantly higher in FHP participants in the experimental, but not the practice session. Mean and maximum BrAC during the second session showed a significant positive correlation with variables describing recent drinking history (e.g. total number of drinks during the last 45 days, bingeing days, and the maximum number of drinks per occasion), connoting a RDH × FHA interaction currently being analyzed. The finding that the effect of familial risk could be detected in a rather small sample suggests that the CASE/Freibier method is sensitive enough to pick up subtle modulators of human alcohol self-administration behavior.

From these studies, we conclude that CASE is practical, safe, and results in consistently higher levels of voluntary brain exposure to alcohol compared to studies employing oral self-administration (see Table 1). In order to increase reliability and the power to detect FHA, it appears that one practice session is useful in order to give participants sufficient confidence to use this unusual procedure according to the instructions. Since CASE results are sensitive to both familial alcoholism and personal recent drinking history in young adults, we

believe that CASE can assess the propensity to develop harmful drinking styles during adolescence.

4.4.3 Test–retest Reliability of CASE and Influence of Recent Drinking History on Self-Administration

The objective of this study was to more thoroughly determine the test–retest reliability of i.v. alcohol self-administration using the Freibier paradigm and to examine the influence of recent drinking history. The study was conducted in 28 healthy 21–45 year-old (17 male and 11 female) social drinkers. Each subject underwent two sessions, between 3 and 30 days apart, following the Freibier protocol described above (Zimmermann et al. 2008; Zimmermann et al. 2009). The maximum allowable breath alcohol concentration was 100 mg%. Participants were instructed to administer alcohol as if they were in a social situation in which they usually drink alcohol. Breathalyzer readings were obtained every 10–15 min to validate the primary measures of self-administration: total number of button presses and both peak and average BrAC during the *ad lib* phase of the session.

There was a high degree of test–retest reliability in self-administration measures across subjects with correlation coefficients ranging from 0.65 to 0.83 (Fig. 8a, b). There was a high level of consistency among measures within session (Fig. 8c). A significant correlation of drinks per drinking day and average BrAC (Fig. 8d) indicates that self-administration behavior in the laboratory setting does reflect drinking behavior in social drinkers. These studies are now being extended to heavier drinking subjects.

4.5 Directions of Future Developments in Intravenous Alcohol Self-Administration

To our knowledge, the CASE system is the only method applying i.v. self-administration of alcohol in humans. CASE is a rather versatile technique and able to accomplish a broad variety of experimental designs, holding promise in two dimensions of alcohol research: phenotyping and drug development. Evaluating the influence of factors such as drinking history, style of drinking, comorbidity, cue-sensitivity, expectation, and sex on ASA can be studied while minimizing pharmacokinetic variability across subjects. Employing the CASE method in the imaging environment should be able to examine the neural correlates of self-administration in non-dependent individuals as well as in individuals at high-risk for developing alcohol dependence. This appears generally feasible since i.v. alcohol administration inside the imaging environment (MRI and PET) is already established, using the PBPK clamping method (Bragulat et al. 2008; Constantinescu et al. 2008; Gilman et al. 2008; Kareken et al. 2010; Ramchandani

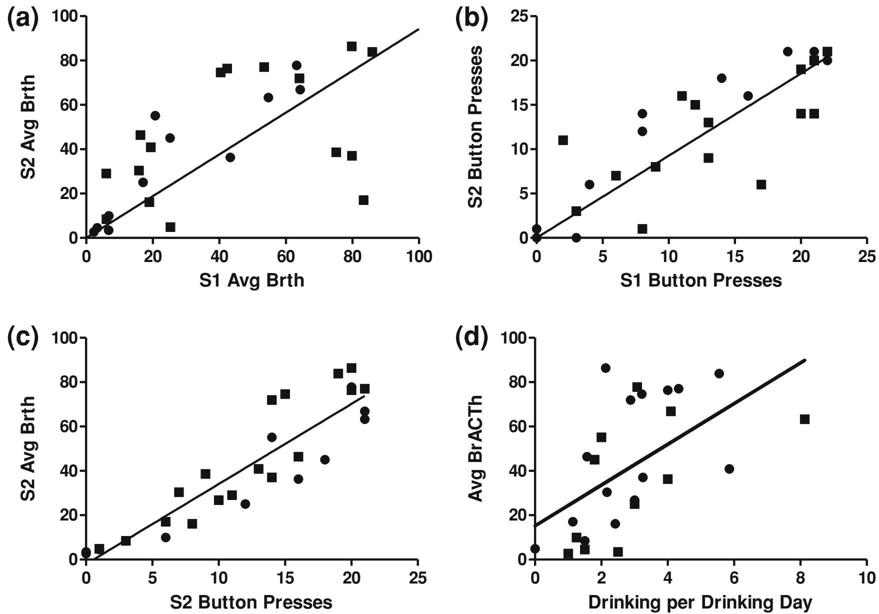

Fig. 8 **a, b** Test–retest reliability of i.v. self-administration measures **c** Consistency among self-administration measures **d** Influence of recent drinking on self-administration in laboratory studies. Lines indicate line of best fit. Circles: females, squares: males

et al. 2010; Yoder et al. 2005, 2007, 2009). Currently already pursued developments of CASE are described in more detail below.

4.5.1 Extension of Operant Response Paradigms to i.v. Self-Administration

Most people work for rewards, and work harder for greater rewards; willingness to work adds an important dependent measure when assessing desire to drink. Much animal work suggests utility of operant response paradigms in pursuit of both drug development and phenotyping applications. In addition, subject safety can be enhanced by shaping the work/reward schedule. Of the different possibilities to make access to alcohol conditional on prior work, the progressive ratio type of reward contingency has been most widely used (see also Sect. 3.2). It implies that the work load required to get access to alcohol progressively increases with each unit used, e.g. 10, 20, 40, and 80 button presses to get access for the first, second, third, fourth etc. self-administration. The outcome measure of such progressive work (PW) experiments is the “breakpoint”, i.e., that point in the cumulative work schedule where the subjects stops to work for more alcohol because it seems not to be worth the further reward of additional incremental exposures to alcohol.

The nature of the work required in PW paradigms is an essential consideration. Most researchers entertain money as a quantitative measure of the desire to drink; mostly everyone works for money, and most drinkers spend money to purchase alcohol. Some studies employ a delayed-discounting scheme; charging the subject for each drink in the form of a deduction from the participation fee to be paid upon discharge. However, charging against a delayed participation fee may not be a suitable approach as it is neither a real-time effort nor a clear alternative reinforcer for alcoholics, and may invoke the relative value of cash vs. credit as a confound. Moreover, the value of a dollar is different for different individuals, and may differ before and after drinking.

Physical exertion is also associated with work, and provides a more immediate and simpler bargain than money. One problem with using physical exertion as the index of willingness to work for alcohol is that different subjects are capable of performing any given task at substantially different rates. Since alcohol, once administered by any route, requires a substantial time to eliminate, frustration may ensue if rate-limiting of accepted work (e.g. counting no more than three button pushes per second) is used to overcome variable capability.

Expectation plays a major role in the nature of the reward offered in PW paradigms. In life, harder or more work is usually rewarded by a bigger, quicker, or more long lasting reward. CASE is capable of providing an identical incremental exposure to alcohol for every subject after sufficient incremental work is performed, but in PW paradigms, progressively more work is rewarded by just "another" exposure. Yet that diminishing return on investment is the key to quantifying the break point, the analysis of which has a rich history in human studies on self-administration of stimulants and opiates. The only published study, to date, that employed a true PW paradigm for alcohol self-administration, used ingestion of 6 gm drinks in return for a geometric increase in the schedule of incremental work required (Barrett et al. 2006; Barrett et al. 2008). These studies demonstrated differential modulations of operant response. Male social drinkers worked harder (higher break point) if the cigarettes they smoked during the two self-administration sessions contain nicotine, but worked less (lower break point) if dopaminergic neurotransmission is dampened by acute tyrosine/phenylalanine depletion (see Table 3).

The CASE system is currently engaged in 3 studies employing a PW alcohol self-administration paradigm using i.v. infusion. Design considerations included the following factors. Subject Safety: continuous performance of rate-limited work cannot raise the BrAC above a preset limit of 200 mg% for the non-treatment-seeking alcoholic study population. Reward: "slopelets" were chosen from many possibilities; when an increment of work is completed, CASE computes the change in the current infusion rate profile that achieves a linear increase in BrAC at 4 mg%/min, to a value that is either 5.0 or 10.0 mg% above the BrAC when the incremental reward began, then switching to a –0.50 mg%/min linear decline until the next work set is completed or equilibration of aBAC and vBAC is achieved and natural elimination ensues. Placebo session vs. alternative reinforcer: At the beginning of any work set, the subject can choose to work for water or alcohol as

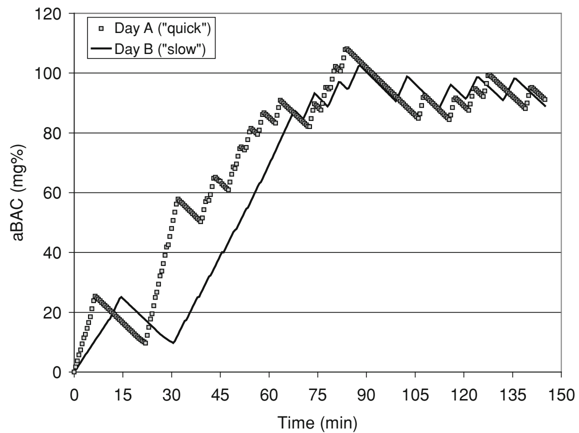

Fig. 9 Time course of BrAC during two CASE experiments in the same subject, comparing "quick" and "slow" rates of BrAC increase. See text for detailed interpretation

the next incremental reward, offering a low-value alternative reinforcer during each session as an alternative to conducting a separate placebo session. Schedule of work: a geometric progression starting at 10 button pushes for the first exposure and escalating to ~2200 to earn the 19th exposure. Craving induction: Current studies employ an alcohol exposure priming phase. CASE paradigms using no priming cues, or introducing gustatory/olfactory cues in place of alcohol exposures for priming, or employing acute stress as cues remain to be evaluated. Dependent measures: Break point, the sequence of button-pushing, and the time course of brain exposure to alcohol are recorded during each session. The CASE system is also being readied for shaping the incremental brain exposure in rat self-administration studies, exploiting the ability to scale the PBPK model across species.

4.5.2 Analysis of the Subject's Intra-Session Adjustments of Brain Alcohol Exposure

During CASE/Freibier experiments, the BrAC is never constant, but either rises (at 3 mg%/min) or falls (at −1 mg%/min), both rates of change being considerably faster than those occurring after oral administration. This means that subjects can quickly bring BrAC into the range they prefer, compensating for BrACs they consider too low (by requesting more often) or too high (by refraining from further alcohol requests for a while) within one single experimental session. This consistency across subjects is a unique feature of CASE and would be impossible with oral self-administration, since the change of BrAC allowed by the interaction of gastrointestinal absorption, redistribution, and hepatic metabolism is too variable for that purpose. On the other hand, these rapid dynamics make it difficult for the subjects to keep their brain alcohol exposure at a stable level over prolonged periods of time. Figure 9 depicts this issue, showing BrAC-time profiles in two experiments in the same subject. Both experiments start with a mandatory priming exposure up to 25 mg%, followed by a waiting period. Actual self-administration

started at 22 min (experiment A, filled squares, quick rise of BrAC) and at 31 min (experiment B, straight lines, slow rise of BrAC). Our interpretation of experiment A is that the subject stepwise approached a level of 110 mg% at 85 min, then decided that this level of alcohol exposure was too high for him and thus refrained from further alcohol requests. Prescribed by CASE settings, BrAC fell approximately 4 times faster than the natural elimination rate, bringing BrAC back to the level where the subject felt comfortable within 20 min (i.e., 90 mg%). Consequently, at 105 min he resumed requesting more alcohol and from thereon managed to keep BrAC constantly at this level with only marginal oscillations. On day B, the BrAC-time course was strikingly similar and again leveled off at 90 mg%.

Visual analysis of the time–BAC profiles in 21 subjects revealed that 8 FHN and 4 FHP subjects likewise produced segments with remarkably stable BrAC extending over at least 40 min, which we call "plateaus". During these plateaus, BrAC oscillated in a sawtooth shape by little more than the inevitable minimal variation of 7.5 mg%, without an upward or downward trend. Of the 9 subjects whose time–BAC profiles did not show such a plateau, 1 was FHN and 8 were FHP. Plateaus occurred significantly more often in FHN than FHP subjects (Fisher's exact test, $p < 0.05$).

We conclude that, although our experimental setup discourages stable levels of BrAC, a subgroup of subjects spontaneously produces constant plateaus of BrAC with only minimal variation, without being instructed to do so. Producing constant plateaus during CASE experiments is only possible if subjects can subtly perceive current alcohol effects, which is their only resource to decide whether or when to request more alcohol. Therefore, the observation that FHP less often produce plateaus suggests that their apperception of alcohol effects is less reliable than in FHN subjects, which might explain why they tend to drink more in real life. Analysis of plateaus appears to be an important new way to evaluate CASE experiments.

4.5.3 Use of i.v. Alcohol Self-Administration for Testing New Drugs to Prevent Relapse in Alcoholics

A series of previous studies established that oral alcohol self-administration is a useful tool to study the effect of pharmacological agents on the rate, magnitude, and pattern of exposure to alcohol, which can serve as a biomarker of the clinical effectiveness of these agents in the potential treatment of alcohol-dependence (see Table 3). CASE methods hold promise to complement these insights by adding the following new aspects: (i) the use of the i.v. method is non-naturalistic so it avoids the influence of olfactory, gustatory, and other cues associated with oral alcohol consumption, which can be both a limitation and an advantage. (ii) CASE yielded higher maximum BrAC levels under baseline conditions, compared to published oral self-administration studies. Specifically, the maximum BrAC in healthy social drinkers (HSD) ranged between 60 and 80 mg% for subjects with low- vs. high-genetic risk for alcoholism (Zimmermann et al. 2009), and ranged up

to 240 mg% in non-treatment-seeking alcoholics (NTSA) (unpublished data), compared to 45 mg% with oral self-administration in both HSD and NTSA (see Table 1). Therefore, CASE might reflect binge/problematic drinking more closely than does oral self-administration. (iii) CASE experiments demonstrated good stability on re-testing, suggesting that within-subjects designs can be reliably used for drug testing. Although test–retest stability was not specifically investigated with oral self-administration, seven of the recent studies testing drug effects did use between-subjects designs. Four of them could substantiate the starting hypothesis. The reason why two others produced a negative and one a mixed result is unclear, but might be related to tenuous stability of the results.

Therefore, the CASE method might serve as a complementary translational tool to screen drugs that have demonstrated the ability to modify free choice or operant self-administration of alcohol in animal models of alcoholism. Studies are underway to evaluate these targets as candidates, including the μ-opioid receptor and the nicotinic acetylcholine receptor. These studies will help demonstrate the usefulness of the CASE paradigm as a method to test the effectiveness of potential treatments for alcoholism.

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Animal Models of Excessive Alcohol Consumption in Rodents

Howard C. Becker

Abstract Numerous animal models have been developed to study excessive alcohol consumption in rodents. Use of such models has played a valuable role in elucidating biological underpinnings and environmental factors that mediate/promote excessive levels of alcohol drinking. A major obstacle in this work has been the need to overcome the natural tendency of rodents to either avoid alcohol or consume it in limited amounts that typically do not produce overt signs of intoxication. A variety of experimental approaches that entail modifying genetic and/or environmental factors have been employed to address this general problem and demonstrate excessive levels of alcohol consumption. Five different approaches that characterize animal models of excessive alcohol consumption are described: models that involve (a) scheduled access to alcohol; (b) scheduled periods of alcohol deprivation; (c) scheduled intermittent access to alcohol; (d) scheduled-induced polydipsia; and (e) dependence and withdrawal experience. Each of the models possesses unique experimental features that engender excessive levels of alcohol consumption. Both advantages and disadvantages for each model are described along with discussion of future challenges to be considered in developing more optimal models. Ultimately, the validity and usefulness of these models will lie in their ability to serve as a platform for studying biological underpinnings and environmental influences that drive increased motivation for alcohol seeking and consumption, as well as evaluation of treatment strategies that effectively reduce excessive levels of alcohol consumption.

H. C. Becker (✉)
Departments of Psychiatry and Neurosciences, Charleston Alcohol Research Center, Medical University of South Carolina, Department of Veterans Affairs Medical Center, 67 President Street, IOP/CDAP-456N, MSC 861, Charleston, SC 29425, USA
e-mail: beckerh@musc.edu

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Contents

1 Introduction 356
2 Models Involving Scheduled Access to Alcohol 357
3 Models Involving Scheduled Alcohol Deprivation 359
4 Models Involving Scheduled Intermittent Alcohol Access 361
5 Models Involving Schedule-Induced Polydipsia 363
6 Models Involving Alcohol Dependence and Withdrawal 365
7 Summary and Future Challenges 368
References 371

1 Introduction

Many neurobiological and environmental factors influence motivation to drink (Grant 1995; Samson and Hodge 1996; Vengeliene et al. 2008; Weiss 2005). At any given time, the propensity to imbibe is generally thought to reflect a balance between the positive reinforcing (rewarding) effects of alcohol (euphoria, anxiolysis) and aversive effects of the drug, typically associated with negative consequences of alcohol consumption (hangover, withdrawal). Memories associated with the rewarding and aversive qualities of alcohol, as well as learned associations between these internal states and environmental stimuli/context, play a critical role in both initiating and regulating intake. Biological (including genetic), environmental, and experiential (learning and memory) factors, along with social forces play a key role in formulating expectations about the consequences of alcohol that, in turn, shape decisions about engaging in drinking behavior. Furthermore, the nature and extent to which these variables are operable not only varies from one individual to another, but also with the stage of development (i.e., adolescence vs. adulthood) and the stage of addiction (i.e., initial experience with alcohol, early problem drinking, and later excessive consumption associated with dependence).

Heavy (excessive) levels of drinking and increased vulnerability to relapse represent hallmark features of alcohol dependence and alcoholism. Hence, development of animal models that incorporate these key behavioral characteristics are critical for advancement of studies aimed at elucidating biological underpinnings and environmental circumstances that engender such maladaptive behavior. Such models are also crucial for identifying new potential therapeutic targets and evaluating efficacy and safety of various treatment strategies.

Numerous experimental approaches have been employed in developing animal models of excessive alcohol self-administration. One of the major obstacles in this work is that rodents typically do not self-administer alcohol in sufficient amounts to

produce overt signs of intoxication. Further, rarely do rodents voluntarily drink alcohol in a manner that results in significant elevation in blood alcohol levels (above legal limits). Thus, a major challenge for the field is to overcome this critical problem so that animal models developed for studying alcohol consumption have greater clinical relevance and, thereby, greater potential for use in both elucidating underlying mechanisms and identifying new and more effective treatment approaches. The following describes several strategies for animal models that have been developed to address the aforementioned shortcomings, with advantages and disadvantages of the various approaches highlighted.

2 Models Involving Scheduled Access to Alcohol

A common approach for studying voluntary alcohol consumption has involved the standard 24 h 2-bottle choice situation. In this model, alcohol solutions of varying concentrations are presented in the home cage along with an alternative fluid (typically water) over a number of days. The main advantage of this approach is that it is relatively simple to implement, it is useful for quickly assessing general avidity for alcohol, and it is a convenient model for examining genetic determinants of the behavior (Crabbe et al. 2010). Further, in a recent study involving analysis of several genetic models, it was shown that the degree of preference demonstrated for alcohol under continuous free-choice conditions corresponded with the relative strength of the reinforcing effects of the drug as measured using operant and other conditioning procedures (Green and Grahame 2008). Nevertheless, a major problem with this unlimited free-access model is that it is difficult to determine whether alcohol intake reaches levels that are physiologically relevant (achieving blood alcohol levels that accompany behavioral signs of intoxication). Limiting access to alcohol is a convenient way to more precisely relate alcohol consumption with resultant blood alcohol levels. Further, since rodents are nocturnal, providing scheduled access to alcohol during the dark phase of their circadian cycle (when eating, drinking, and general activity is at the highest levels) facilitates greater alcohol consumption.

Limited access to alcohol restricted to the dark phase of the circadian cycle has been used to model binge-like drinking (Crabbe et al. 2011a) as well as demonstrate escalated drinking in dependent subjects (see below). In the former case, a mouse model sometimes referred to as “drinking in the dark” was developed with the goal being to restrict access to alcohol such that intake over a defined period of time produces blood alcohol levels above the U.S. legal limit of intoxication ($\geq$0.08 g/dL)—thereby satisfying the clinical criteria for binge-like drinking. In this model, mice are offered a single bottle of alcohol (20% v/v) for 2 h starting 3 h after the dark phase begins for 3 days followed by a 4th day when access is extended to 4 h. This scheduled alcohol access produced significant consumption on the final day of this 4-day procedure in C57BL/6 mice, with resultant blood alcohol levels typically reaching $\geq$0.10 g/dL (Rhodes et al. 2005). Not surprisingly, alcohol intake in this model differed substantially across genotypes (inbred mouse strains) and, importantly, drinking in C57BL/6 mice produced observable signs of intoxication as indexed by

measures of motor incoordination (Rhodes et al. 2007). When the model incorporated a 2-bottle choice situation (water available as the alternative fluid), reduced alcohol intake and resultant blood alcohol levels have been reported (Phillips et al. 2010; Rhodes et al. 2007). Nevertheless, the model engenders relatively high alcohol intake within a short period of time and the high level of drinking does not appear related to motivation for obtaining calories contained in the alcohol (Lyons et al. 2008). Extending the single-bottle procedure (2 h sessions) for 14 days produced faster rates of consumption (more drinking during the early portion of the drinking sessions) and tolerance to the ataxic effects of alcohol (Linsenbardt et al. 2011). This model has also been effectively used to study consequences of alcohol binge-like exposure in utero (Boehm et al. 2008) and during adolescent periods of development (Metten et al. 2011).

Recently, Crabbe et al. initiated a selective breeding program using a modified version of this model to create mice that drink a sufficient amount of alcohol that produces high blood alcohol levels along with behavioral signs of intoxication. In this work, mice from a genetically heterogeneous stock were tested in a 2-day single-bottle (20% alcohol) paradigm (2 h access the first day and then 4 h access the next day, both during the early part of the dark cycle). Male and female mice were selected for breeding based on the highest blood alcohol levels achieved following the 4-h drinking session. This genetic selection was repeated over several generations such that average resultant blood alcohol levels increased from an initial value of approximately 0.03 g/dL (prior to selective breeding) to ≥0.10 g/dL (Crabbe et al. 2009). This High Drinking-in-the-Dark (HDID-1) selected line also consumed significantly more alcohol than the control line from which they were selected, even though the selection was based on blood alcohol levels (not amount of alcohol consumed). Interestingly, HDID-1 mice from the 13–17th selected generations consumed similar amounts of alcohol and other tastants (sucrose, saccharin, quinine) as the control line when the solutions were presented under continuous (24-h) access conditions. However, greater intake was noted in the HDID-1 mice when preference testing was extended for a longer period of time under limited access conditions (Crabbe et al. 2011b). A second replicate line (HDID-2) has since been created and results appear to be following a similar pattern of selection (Crabbe et al. 2010).

A variation of this mouse model has been used to study binge-like drinking in rats selectively bred for high alcohol preference (P rats). Earlier work showed that P rats consumed more alcohol in a 2-bottle free-choice situation (10% alcohol vs. water) when access was scheduled over four 1-h periods (each separated by 2 h) during the dark cycle compared to when the alcohol was available continuously for the equivalent 4-h period (Murphy et al. 1986). Building on these results, this multiple-scheduled-access model was modified by offering P rats access to three fluids (water vs. 15% alcohol vs. 30% alcohol) over three 1-h access periods during the dark phase of the circadian cycle. Over several weeks alcohol consumption in this model was shown to register significant blood alcohol levels (>0.08 g/dL) as well as behavioral signs of intoxication (motor impairment) (Bell et al. 2011; McBride et al. 2010).

3 Models Involving Scheduled Alcohol Deprivation

Animals with a long history of daily access to alcohol display a transient, yet robust increase in voluntary alcohol consumption and preference when alcohol is reintroduced after a period of deprivation. This alcohol deprivation effect was first formally described in rats (Sinclair and Senter 1968), but has also been demonstrated in mice (Salimov and Salimova 1993; Salimov et al. 2000; Tambour et al. 2008), monkeys (Kornet et al. 1990; Sinclair 1971), and humans (Burish et al. 1981). Most studies have examined the phenomenon in rats using 2-bottle choice continuous access models. Increased alcohol drinking has been noted after relatively brief periods of deprivation (<24 h) as well as following longer (several weeks) deprivation intervals (Sinclair and Li 1989). The alcohol deprivation effect has also been demonstrated using limited access operant self-administration procedures in rats (Heyser et al. 1997; Holter et al. 1997) and mice (Sparta et al. 2009). However, there were no effects of deprivation on alcohol intake reported in a study using a modified (sipper-tube) self-administration procedure (Samson and Chappell 2001).

The alcohol deprivation effect has been demonstrated in outbred rat strains such as Wistar (Vengeliene et al. 2003) and Long–Evans (Sinclair and Tiihonen 1988). Similarly, an alcohol deprivation effect has been reported in rats selectively bred for high alcohol preference (P rats) under free-choice continuous access and limited access operant paradigms (McKinzie et al. 1998; Sinclair and Li 1989; Vengeliene et al. 2003). However, a robust increase in alcohol consumption following a period of deprivation has not been reliably observed in other rat lines selectively bred for high alcohol preference, including the Alko Alcohol-Accepting (AA) rats (Sinclair and Li 1989; Sinclair and Tiihonen 1988) and the Indiana High Alcohol Drinking (HAD) rats (Rodd-Henricks et al. 2000b). The Sardinian P (sP) rats, which were generated using the same selection criteria as for the Indiana P rats, showed a fairly modest increase in alcohol intake that was very brief in duration (Agabio et al. 2000). Collectively, these data do not support a consistent relationship between selection for high alcohol preference/intake and expression of a robust alcohol deprivation effect.

Although the alcohol deprivation effect has been viewed as a model for alcohol relapse and craving, there are some drawbacks related to the model. One concern relates to the specificity of the phenomenon, since exaggerated intake of other rewarding tastants (e.g., sucrose, saccharin) can be demonstrated in rats following a period of deprivation (Avena et al. 2005; Wayner et al. 1972). As noted above, the increase in alcohol intake after short or long periods of deprivation is typically short-lived, with intake returning to baseline (pre-deprivation) levels in a few days. However, when P rats are given concurrent access to several alcohol concentrations (10, 20, and 30%) along with water, the alcohol deprivation effect was shown to be more robust and more durable (Rodd-Henricks et al. 2001). Further, this same manipulation was reported to produce an alcohol deprivation effect in HAD rats even though these animals do not show such an effect when a single alcohol concentration is offered in a free-choice situation (Rodd et al. 2004).

While enhanced alcohol intake following a single deprivation period has been shown to be a transient effect, repeated deprivation experience has been shown to produce longer lasting increases in alcohol consumption. After long-term free access to several alcohol solutions, repeated "forced" abstinence periods resulted in progressively greater enhancement of alcohol intake, a shift in preference for higher alcohol concentrations, and longer lasting deprivation effects in Wistar rats (Spanagel and Holter 1999, 2000) and P rats (Rodd-Henricks et al. 2000b, 2001). Additionally, concurrent access to multiple concentrations of alcohol along with exposure to repeated cycles of deprivation produced significant increases in alcohol consumption in HAD rats, a genotype that does not readily exhibit an alcohol deprivation effect following a single period of deprivation (Rodd et al. 2009; Rodd-Henricks et al. 2000a). Using a similar experimental strategy involving multiple alcohol concentrations (0, 5, 10, and 15%) and several cycles of deprivation, increased alcohol consumption was demonstrated over repeated episodes of re-exposure to alcohol in rats selectively bred for low alcohol preference and drinking (NP and LAD rats) (Bell et al. 2004). This suggests that genetic selection for low alcohol preference/consumption can be overcome by experimental parameters that ordinarily engender expression of a more robust alcohol deprivation effect. Interestingly, offering several alcohol concentrations and repeated cycles of deprivation did not alter the magnitude or duration of the relatively brief and modest alcohol deprivation effect in sP rats (Serra et al. 2003).

In addition to enhancing the alcohol deprivation effect under 24-h free-choice conditions, repeated episodes of deprivation augmented and prolonged oral alcohol self-administration using operant conditioning procedures (Oster et al. 2006; Rodd et al. 2003; Spanagel and Holter 2000). Further, this effect demonstrated in Wistar, P, and HAD rats was shown to be accompanied by an apparent enhancement of the reinforcing efficacy of alcohol, as indexed by higher breakpoint values under progressive ratio testing procedures (Oster et al. 2006; Rodd et al. 2003; Spanagel and Holter 2000). In a long-term drinking model involving several months of free-choice alcohol access and multiple episodes of deprivation, Wistar rats not only increased alcohol intake and demonstrated a progressive shift in preference for higher previously less preferred alcohol concentrations, but these rats also exhibited less sensitivity to the otherwise unfavorable adulteration of alcohol with quinine (Spanagel et al. 1996). This latter effect has been suggested to reflect more compulsive aspects of drinking that develops as a function of long-term access to alcohol with repeated intervening periods of deprivation (Spanagel 2009).

Fewer studies have systematically studied the alcohol deprivation model in mice. In one study, the effects of repeated deprivation cycles on alcohol intake under 2-bottle choice (10% alcohol vs. water) continuous access conditions were shown to differ in substrains of C57BL/6 mice (Khisti et al. 2006). Repeated 4-day deprivation periods initially produced a robust alcohol deprivation effect in C57BL/6NCrl mice, but the transient increase in intake diminished in magnitude over successive deprivation cycles. In contrast, alcohol consumption did not significantly change following single or multiple cycles of deprivation in C57BL/6J mice. In a modified version of the alcohol deprivation effect, C57BL/6J mice showed increased alcohol

intake following repeated weekly deprivation periods of 6 days (alcohol was reinstated 1 day each week). However, this effect was abolished with a longer (2 week) deprivation period (Melendez et al. 2006). Although relatively few studies have examined the alcohol deprivation effect in mice, single or multiple deprivation periods have not reliably produced enhanced alcohol drinking when alcohol is offered in the home cage under limited access conditions (Becker, unpublished observations).

4 Models Involving Scheduled Intermittent Alcohol Access

A model that has recently gained popularity and that engenders a high level of alcohol consumption involves chronic intermittent access to alcohol. In this model, inherent in the scheduled intermittency of free access to alcohol are repeated periods of abstinence. Although the model is similar to the paradigm described above involving repeated periods of deprivation, in this case the periods of alcohol access and deprivation are relatively short (days rather than weeks), thereby accelerating the pace at which excessive levels of alcohol intake can be established. This chronic intermittent access procedure was first described to produce increased drinking in rats when alcohol was provided on a continuous basis for 2 days with intervening 2-day abstinence periods (Wayner et al. 1972) or for 24 h every other day (Wise 1973). In a more recent study, free access to 20% alcohol was offered in a 2-bottle choice situation (with water) for 24 h 3 days a week (with no more than 2 days of abstinence between access days). Within 5–6 drinking sessions, alcohol consumption increased from baseline levels of about 2 g/kg/24-h to approximately 5–6 g/kg/24-h in Long–Evans rats (Simms et al. 2008). A similar outcome was reported in another study where Long–Evans rats exposed to the same procedure displayed progressively increased consumption and preference for 20% alcohol over 20 drinking sessions (Carnicella et al. 2009). This escalation of drinking along with increased preference for alcohol was also demonstrated in Wistar rats (Simms et al. 2008), although another study using a 3-bottle choice situation (water vs. 5% vs. 20%) reported a two to three fold difference in the change in alcohol intake and preference depending on the supplier of Wistar rats (Palm et al. 2011). The escalation of intake in Long–Evans and Wistar rats registered significantly elevated blood alcohol levels in samples taken after the first 30 min of the drinking sessions, with several subjects attaining levels >0.08 g/dL (Carnicella et al. 2009; Simms et al. 2008). Increased alcohol consumption has also been noted in Sprague–Dawley rats following the 2-bottle (water vs. 20% alcohol) intermittent access paradigm (Bito-Onon et al. 2011), but the effect may only be observed in a portion of the animals (Moorman and Aston-Jones 2009).

Similar studies have been recently conducted in mice. For example, Melendez (2011) reported that adult C57BL/6J mice provided 24 h access to a 2-bottle choice of 15% alcohol and water consumed significantly more alcohol when it was presented every other day in comparison to mice that received continuous access to alcohol every day. That is, initial alcohol intake (6–7 g/kg/24-h) escalated to

14–15 g/kg/24-h over 7 drinking sessions in the intermittent access group while intake increased to 8–9 g/kg/24-h in the continuous access group. A large portion of the alcohol was consumed within the first 6 h (of the dark phase), and the increased level of drinking in the intermittent group reverted to lower baseline levels of intake when a continuous access schedule was implemented (Melendez 2011). In another study, C57BL/6J mice were first acclimated to increasing concentrations of alcohol and then maintained on a 24 h 2-bottle choice (20% alcohol and water) regimen, with access scheduled either every other day or continuously everyday. Over the course of 4 weeks, alcohol consumption was >20 g/kg/24-h in the intermittent access group compared to ~16 g/kg/24-h for the continuous access group (Hwa et al. 2011). This effect was even greater in female C57BL/6J mice, and extending intermittent access for 16 weeks in the male subjects resulted in mild expression of withdrawal-related hyperexcitability. Also, intake over the first 2 h in a single bottle test with 20% alcohol was greater in mice with intermittent compared to continuous access, and this greater intake resulted in higher blood alcohol levels (Hwa et al. 2011). However, using similar procedures, others have not observed this large a difference in intake between mice offered alcohol in an intermittent versus continuous fashion (J.C. Crabbe, personal communication; S.E. Bartlett, personal communication). At present, it is unclear what factors may contribute to this discrepancy in results.

In addition to home-cage drinking, this intermittent alcohol access model has also been extended to oral alcohol self-administration behavior using operant conditioning procedures. For example, Long–Evans rats were shown to vigorously respond to self-administer 20% alcohol when operant sessions scheduled every other day were gradually reduced from overnight to 30 min in duration (Simms et al. 2010). The increased amount of alcohol self-administered resulted in significant elevation of blood alcohol levels, with average values ~0.06 g/dL and several rats registering blood alcohol levels above 0.10 g/dL (Simms et al. 2010). In another study, prolonging the intermittent access schedule for several months not only increased home-cage alcohol drinking, but also transferred to increased operant self-administration of oral alcohol in Wistar rats (Hopf et al. 2010). Further, rats maintained on the intermittent access schedule to 20% alcohol for 3–4 months demonstrated resistance to quinine adulteration of alcohol in home-cage drinking and operant responding, but this effect was not observed in rats with a history of intermittent alcohol access for only 1.5 months (Hopf et al. 2010).

A few studies have examined drinking in this intermittent access model in rats selectively bred for high alcohol preference. For instance, P rats were shown to exhibit an increase in alcohol intake under conditions in which 24 h free-choice (20% alcohol vs. water) access was given every other day. However, this increase in alcohol consumption from an average baseline level of intake (4–5 g/kg/24-h) to 6–7 g/kg/24-h over 20 drinking sessions was relatively modest compared to the escalation of intake exhibited in Long–Evans and Wistar rats reported in the same study (Simms et al. 2008). In contrast, using a similar 2-bottle choice (20% alcohol vs. water), every other day scheduled access paradigm, the Sardinian P (sP) rats showed robust escalation of drinking from baseline intake levels at ~5 to 9–10 g/kg/24-h over 20 drinking sessions (Loi et al. 2010).

This increase was also noted during the first hour of access during the dark phase, with intake rising from baseline levels of ~0.5 to 1.5–2.0 g/kg. Alcohol consumption in sP rats given intermittent access significantly exceeded intake registered in sP rats that were given the same alcohol solution (20% vs. water), but in a continuous access pattern. After 10 drinking sessions, consumption in the intermittent access group produced behavioral signs of intoxication, as measured by motor impairment in a rotarod task. Additionally, these rats exhibited resistance to effects of quinine adulteration of alcohol as well as competing effects of concurrent access to saccharin (Loi et al. 2010). It is interesting that sP rats are very responsive to this chronic intermittent access procedure in which relatively short periods of access and abstinence (deprivation) are repeatedly alternated while the Indiana P rats (but not sP rats) display robust escalation of drinking in a model of repeated deprivations where access and deprivation periods are longer in duration (Rodd-Henricks et al. 2001; Serra et al. 2003). An explanation for this discrepancy is not readily apparent at present (Loi et al. 2010).

5 Models Involving Schedule-Induced Polydipsia

Animals have been shown to engage in excessive drinking behavior when delivery of food reinforcement is scheduled in an intermittent fashion (typically a fixed time interval) that is not under the animal's control (Falk 1961). This adjunctive behavior (excessive drinking) is displayed as a consequence of and in relation to another behavior that is evoked by environmental change (eating small amounts of food delivered in a scheduled manner that is not determined by the animal). The term schedule-induced polydipsia refers to the excessive nature of adjunctive drinking under these conditions, which greatly exceeds fluid intake that would occur if the same total amount of food was presented all at once.

When an alcohol solution is the available fluid, this schedule-induced polydipsia results in excessive levels of alcohol consumption (10–14 g/kg/24-h) in rats that leads to dependence, as evidenced by overt signs of withdrawal when the alcohol is removed (Falk and Samson 1975; Falk et al. 1972). There is some controversy about whether continuous access to alcohol is required to induce dependence. For example, when access to alcohol and the fixed time schedule of food reinforcement was restricted such that drinking produced one or two daily peaks in blood alcohol levels, there was no evidence of dependence (Samson and Falk 1975). However, in another study daily 3-h sessions for a few months was reported to be sufficient to produce dependence (Tang and Falk 1983). In a more recent study, a schedule-induced polydipsia procedure was used to assess alcohol consumption in rats selectively bred for high and low alcohol preference (Gilpin et al. 2008a). Across a number of alcohol concentrations, P rats and one of the replicate lines of HAD rats showed greater water and alcohol intake compared to their non-preferring counterparts (NP and LAD-2 rats). In all cases, blood alcohol levels were positively correlated with alcohol intake after the 1-h sessions, with many rats registering levels >0.08 g/dL (Gilpin et al. 2008a).

Schedule-induced polydipsia procedures have also been used to examine alcohol drinking in mice. In an early study involving outbred (ICR-DUB) female mice, four daily 1-h sessions (each separated by 6 h) produced high levels of drinking in mice given access to 6% alcohol (14–20 g/kg/day) or 10% alcohol (17–25 g/kg/day). In both cases, this level of intake over 7 days was not sufficient to produce significant signs of withdrawal following the scheduled access phase of the study (Ogata et al. 1972). Over 20 daily 1-h sessions, the alcohol-preferring C57BL/6J inbred strain consumed a substantial amount of 5% alcohol ($\sim$5 g/kg) relative to their initial intake ($\sim$1 g/kg), and several mice evidenced blood alcohol levels >0.20 g/dL. In contrast, the non-preferring DBA/2J inbred strain showed only very modest increase in alcohol consumption under the same schedule conditions (Mittleman et al. 2003).

Advantages of this model are that animals consume large quantities of alcohol orally and on a voluntary basis (Falk and Tang 1988). Disadvantages of this approach include lack of specificity of the effect since polydipsia can be seen when other fluids are made available, and the fact that animals are typically maintained on a food-restricted diet. This latter issue raises concern about whether motivation to drink alcohol is related to its pharmacological effects or its caloric content. Another shortcoming is that when the schedule of intermittent reinforcement is relaxed, alcohol consumption reverts to control levels in rats (Tang et al. 1982). That is, elevated alcohol drinking does not endure under free-choice conditions even though the animals consumed large amounts of alcohol when it was available under intermittent schedules of food reinforcement.

However, inasmuch as such schedules that induce adjunctive behaviors are stressful (Falk 1971; Lopez-Grancha et al. 2006), it may be that studies in rodents have not utilized experimental parameters that are optimal for establishing the negative reinforcing effects of alcohol (Kathy Grant, personal communication). That is, while schedule-induced polydipsia procedures are effective in establishing the positive reinforcing effects of alcohol (Meisch 1975), experimental conditions that facilitate association of alcohol consumption with stress relief (escape from the onerous nature of the intermittent, response non-contingent schedule of food delivery) may be required for producing long-lasting elevated drinking. Interestingly, sustained excessive levels of alcohol consumption have been demonstrated in studies conducted by Grant et al. (2008) where schedule-induced polydipsia procedures are employed to induce alcohol drinking in non-human primates. Further, the pattern of drinking during the induction phase was shown to predict the degree of heavy drinking in male cynomolgus monkeys during a subsequent 12-month continuous free-choice access period. Excessive alcohol consumption during this free-access period produced behavioral signs of intoxication in many of the subjects. Additionally, extending the open-access period to more than 2 years along with intervening periods of abstinence not only produced sustained excessive levels of alcohol consumption, but also resulted in functional (synaptic) and morphological adaptations in the putamen (Cuzon Carlson et al. 2011). Thus, the schedule-induced polydipsia procedure has proven to be effective and integral to this monkey model of heavy drinking that captures many of the features of alcoholism in humans.

6 Models Involving Alcohol Dependence and Withdrawal

Alcohol dependence has long been postulated to play a significant role in fostering and perpetuating excessive drinking (Cappell and LeBlanc 1981; Grant 1995). Early studies generally yielded equivocal findings (Begleiter 1975; Deutsch and Koopmans 1973; Hunter et al. 1974; Myers et al. 1972; Numan 1981; Samson and Falk 1974; Schulteis et al. 1996; Winger 1988), but this was most likely due to procedures that did not sufficiently establish the positive reinforcing effects of alcohol prior to dependence induction. Further, in most of these early studies subjects had minimal opportunities to associate alcohol drinking with its withdrawal-alleviating consequences and, hence, did not optimize the development of the drug's negative reinforcing capacity (Meisch 1983; Meisch and Stewart 1994). Incorporating these procedural considerations, more recent studies have been successful in linking dependence models with self-administration procedures (Becker 2008; Becker et al. 2011).

Indeed, in the past decade numerous studies involving mice and rats have demonstrated escalated alcohol consumption using home-cage free-choice models and operant conditioning procedures. In most cases, dependence has been induced by administering alcohol vapor via inhalation chambers, with the chronic alcohol exposure delivered in an intermittent pattern such that multiple withdrawal episodes are experienced. For example, rats exposed to chronic alcohol treatment interspersed with repeated episodes of withdrawal consumed significantly more alcohol than controls under free-choice unlimited (24 h/day) access conditions (Rimondini et al. 2002, 2003; Sommer et al. 2008). Similar results have been reported in mice using a dependence model involving repeated cycles of chronic intermittent alcohol vapor exposure and with voluntary alcohol consumption assessed using a limited access (2 h/day) schedule (Becker and Lopez 2004; Dhaher et al. 2008; Finn et al. 2007; Lopez and Becker 2005). The intensity of repeated chronic alcohol exposure (producing high and sustained blood alcohol levels) was shown to be critical in favoring escalation of alcohol consumption in the model (Griffin et al. 2009a). Further, the effect appears specific to alcohol because repeated cycles of chronic intermittent alcohol exposure did not produce alterations in water intake or consumption of highly palatable fluids such as sucrose and saccharin (Becker and Lopez 2004; Lopez et al. 2011). This suggests that the increase in alcohol consumption is not a non-specific effect related to a general need to hydrate with fluids or increase caloric intake. Additionally, studies using operant conditioning procedures have demonstrated increased alcohol self-administration in mice (Chu et al. 2007; Lopez et al. 2008) and rats (Funk and Koob 2007; Gilpin et al. 2008b, c, 2009; O'Dell et al. 2004; Richardson et al. 2008; Roberts et al. 1996, 2000) with a history of repeated chronic alcohol exposure and withdrawal experience.

Enhanced alcohol responding/intake in dependent animals occurred well beyond acute withdrawal, and escalation of alcohol self-administration was especially facilitated when dependence induction involved delivery of chronic alcohol in an

intermittent rather than continuous fashion (Lopez and Becker 2005; O'Dell et al. 2004). This latter finding suggests that elevated alcohol self-administration does not merely result from long-term alcohol exposure per se, but rather, repeated withdrawal experience plays a critical role in driving enhanced motivation for alcohol. Additionally, with an increased number of chronic alcohol exposure/ withdrawal cycles, up-regulated alcohol intake was further augmented and sustained for a longer period of time (several weeks) following final withdrawal compared to intake in a separate group of non-dependent mice (Lopez and Becker 2005). Further, analysis of the temporal pattern of alcohol consumption revealed that dependent mice not only consumed more alcohol than non-dependent animals over the entire 2-h access period, but the rate of consumption was faster and progressively increased over successive withdrawal test periods (Griffin et al. 2009b).

In both mice and rats, the escalation of alcohol self-administration following repeated cycles of chronic intermittent alcohol exposure was reported to be associated with significantly higher resultant blood alcohol levels compared to that achieved by more modest and stable levels of intake in non-dependent animals (Becker and Lopez 2004; Roberts et al. 2000). Additionally, the faster rate of alcohol intake and greater overall amount consumed exhibited by dependent mice has been shown to result in significantly higher peak and more sustained alcohol concentrations measured in brain compared to levels achieved from consumption of alcohol in non-dependent animals (Griffin et al. 2009b). Moreover, greater voluntary alcohol consumption in dependent mice produced brain alcohol concentrations that approximated those levels experienced during chronic intermittent alcohol exposure that rendered the subjects dependent in the first place. While it is tempting to speculate that dependent animals display increased voluntary alcohol drinking behavior to attain blood and brain alcohol levels in a range consistent with sustaining dependence, the extent to which resultant brain alcohol concentrations play a role in driving as well as perpetuating enhanced alcohol drinking in dependent animals remains to be determined.

Despite the growing and convergent body of evidence indicating that rodent models of dependence involving chronic intermittent alcohol exposure produce robust escalation of voluntary alcohol consumption, the mechanisms underlying enhanced motivation to imbibe in the context of dependence are not fully understood. As noted above, mechanisms that govern the regulation of drinking behavior involve complex and dynamic processes. An interplay among numerous biological and environmental factors influence motivational effects of alcohol, and these may change as the subject gains more experience with the drug (Cunningham et al. 2000). Alcohol dependence may be characterized as an allostatic state fueled by progressive dysregulation of motivational processes and neural circuitry controlling intake (Becker 2008; Heilig et al. 2010; Koob 2003). Such neuroadaptations may play a role in enhancing the rewarding effects of alcohol, thereby fostering the transition from regulated alcohol use to uncontrolled, excessive levels of drinking. Additionally, the potential for alcohol to alleviate negative affect and other symptoms of withdrawal serves as a powerful motivational force that likely promotes and sustains high levels of drinking (Becker 2008; Heilig et al. 2010).

Indeed, there is evidence to suggest that chronic intermittent alcohol exposure enhances the rewarding effects of the drug. Studies employing operant self-administration procedures have demonstrated augmented motivation to self-administer alcohol (increased responding and consumption) in alcohol-dependent mice (Chu et al. 2007; Lopez et al. 2008) and rats (Gilpin et al. 2008c, 2009; O'Dell et al. 2004; Roberts et al. 1996, 2000). Further, employing progressive ratio schedules, it was demonstrated that the amount of work mice (Lopez et al. 2008) and rats (Brown et al. 1998) were willing to expend in order to receive alcohol reinforcement was significantly increased following repeated cycles of chronic alcohol exposure/withdrawal experience. Also, animals with a history of alcohol dependence have been shown to exhibit exaggerated sensitivity to the effect of alcohol-related cues and stressors to enhance alcohol-seeking behavior (Gehlert et al. 2007; Liu and Weiss 2002; Sommer et al. 2008). These findings suggest that the reinforcing value of alcohol may be enhanced and subjects may be rendered more vulnerable to relapse as a consequence of experiencing repeated opportunities to self-administer alcohol in the context of chronic intermittent exposure to the drug.

At the same time, another factor that could play an important and permissive role in excessive drinking is the development of tolerance to the aversive effects of alcohol. Tolerance has long been viewed as playing an important role in the regulation of alcohol self-administration behavior (Cicero 1980; Deitrich et al. 1996; Kalant 1996, 1998; Rigter and Crabbe 1980; Suwaki et al. 2001). Thus, as a consequence of chronic alcohol exposure, the development of tolerance to the aversive effects of alcohol (which ordinarily temper amount consumed) may serve as a permissive factor, enabling higher levels of drinking. Recent evidence indicates that repeated cycles of chronic intermittent alcohol exposure in mice not only produces escalation of voluntary drinking, but also reduced sensitivity (tolerance) to the aversive effects of alcohol in the same subjects, as determined by a conditioned taste aversion procedure (Lopez et al. 2011). This reduced sensitivity to alcohol-induced conditioned taste aversion could not be attributed to pharmacokinetic factors, and it could not simply be explained by a general learning deficit since both dependent and non-dependent mice exhibited similar learned aversion to a non-alcohol noxious stimulus (lithium chloride). In another study, rats with a history of repeated cycles of chronic alcohol exposure and withdrawal were reported to exhibit long-lasting tolerance to the sedative/hypnotic effects of alcohol (Rimondini et al. 2008). Additionally, using operant discrimination procedures, it was found that the ability to detect (perceive) the subjective cues associated with alcohol intoxication was diminished during withdrawal from chronic alcohol exposure, and this tolerance effect was greater in mice that experienced multiple withdrawals during the course of the chronic alcohol treatment (Becker and Baros 2006). Thus, reduced sensitivity to feedback about the intoxicating effects of alcohol along with reduced sensitivity to the aversive effects of the drug may serve a permissive role in enabling greater alcohol consumption associated with dependence.

Collectively, these data support the notion that with prolonged alcohol exposure, the relative balance between rewarding/reinforcing and aversive properties of alcohol is shifted away from aversion in favor of reinforcement. Thus, the combination of enhanced rewarding effects (through both positive and negative reinforcement) along

with reduced sensitivity (tolerance) to the aversive qualities of alcohol intoxication may, in large part, drive excessive drinking associated with dependence. Elucidating neurobiological mechanisms underlying changes in sensitivity to both the rewarding and the aversive effects of alcohol is key to understanding motivational processes that are critical for regulating and controlling alcohol consumption, as well as adaptations in such processes that mediate transition to uncontrolled, harmful levels of drinking characteristic of dependence.

7 Summary and Future Challenges

Numerous animal models have been developed and used to study excessive alcohol consumption in rodents. A common goal of this work has been to overcome the natural tendency of rodents to either avoid alcohol or consume it in limited amounts that typically do not produce overt signs of intoxication. A variety of experimental approaches that entail modifying genetic and/or environmental factors have been employed to address this general problem and demonstrate excessive levels of alcohol consumption. Here, we provide a general overview of five different models with unique experimental features that have been commonly employed to study excessive alcohol consumption in laboratory rodents.

For the most part, models described in this chapter incorporate a number of procedural variables that include manipulating scheduled access to alcohol (time of day, duration, frequency), periods of time when access to alcohol is withheld, and history of alcohol exposure. As noted above, each of the models possesses distinct advantages and disadvantages. Models that involve scheduled access to alcohol exploit the natural tendency of nocturnal rodents to engage in consummatory behavior during the dark phase of the circadian cycle along with restricting the duration of access so as to enhance the measured relationship between amount of alcohol consumed and resultant blood alcohol levels. This approach has provided a good model of binge-drinking, where alcohol intake is associated with high blood alcohol levels and behavioral signs of intoxication. Other positive features include the simplicity of executing the procedure and the rapid manner in which high levels of drinking are attained. While intake in this model does not appear related to the caloric value of the alcohol, restriction of access to the early part of the active phase of the circadian cycle (when rodents consume a large proportion of their daily food intake) raises the question of whether postprandial mechanisms (i.e., thirst) may contribute to the high level of drinking. This issue has not been systematically addressed. Further, effects of more extensive exposure using this model (i.e., repeated binge-drinking opportunities over several weeks) has not been extensively studied. Hence, it is not clear whether underlying mechanisms reflect those that drive drinking in subjects with histories of more extensive alcohol exposure. This may be important for clinical relevance and utility of the model in evaluating potential treatments that temper such high levels of drinking over a longer period of time.

The second model described involves imposing extended periods of time when access to alcohol is withdrawn following relatively long periods of free access. From an anthropomorphic standpoint, this "alcohol deprivation" model is appealing in its relation to the construct craving, which has been shown to be a significant factor that drives relapse. However, there are questions about specificity of the effect and the transient nature of the increase in drinking. The latter concern has more recently been addressed experimentally by providing access to several alcohol solutions of varying concentration and repeating the deprivation experience. Unfortunately, studies that address why such experimental variables enhance the alcohol deprivation effect are generally lacking. Further, the biological mechanisms underlying the phenomenon are generally unknown. It will also be important to determine whether pharmacological interventions that reduce this deprivation-induced elevation in drinking are also effective in the more robust situation where the effect is longer lasting after several deprivation cycles.

A third model that engenders high levels of alcohol consumption involves relatively frequent alternating periods of access and no-access to the drug. Models involving intermittent access to alcohol (typically every other day) in a free-choice situation have recently enjoyed a resurgence of interest and, while not universally observed, these models have for the most part been demonstrated to effectively produce significant increases in alcohol consumption in rats and mice. Genetic factors have been shown to exert influence on escalated drinking in this model, but this has mostly been restricted to analysis of different rat genotypes. In many cases, individual variability has been reported with regard to the magnitude of the effect. However, it is uncertain as to why some animals display escalation of drinking while others do not in a given study. Further, an explanation for why intermittency of access to alcohol drives increased drinking is not readily apparent and there have been few studies aimed at understanding mechanisms underlying the phenomenon. Systematic analysis of experimental parameters (e.g., duration of access, frequency of access, predictability of access) that favor escalation of drinking (or resistance to such increases) is critical in guiding future mechanistic studies. It is interesting that some studies have demonstrated that drinking returns to lower levels once alcohol is presented in a continuous manner. This calls into question the durability of changes that may underlie the escalation effect and this is something to consider when pharmacological agents are tested in such models. Clearly, more research is needed to better understand mechanisms underlying escalation of drinking and whether prolonged exposure to intermittent schedules of access produce enduring adaptations that reflect increased motivation to drink.

Another model used to demonstrate excessive levels of alcohol consumption involves use of operant conditioning equipment that provides access to alcohol while enabling regularly scheduled delivery of small amounts of food. This schedule-induced polydipsia model has been shown to produce high levels of alcohol intake that lead to signs of intoxication and dependence. However, as pointed out above, a main drawback is that excessive levels of alcohol intake are not sustained once the unique schedule of food delivery is suspended. Thus, the durability of the effect is a concern, and few studies have examined biological underpinnings of the phenomenon. This is

especially problematic for studies aimed at elucidating biological mechanisms that underlie sustained increase in motivation for alcohol consumption and how such changes can be effectively targeted with pharmacological agents. Future studies are needed to examine experimental parameters of the model that facilitate promoting the negative reinforcing effects of the drug, as this may engender more long-lasting effects on alcohol drinking behavior. Also, the role of stress associated with this procedure (the regularly scheduled delivery of small amounts of food that is not controlled by the subject) has not been fully explored as it relates to the outcome of excessive alcohol intake. The schedule-induced polydipsia model has been successfully employed in monkey models as an induction procedure that leads to increased alcohol consumption in a free-choice situation. It is not clear whether this is a species-specific effect or whether optimal parameters of the model have not been identified in rodent studies that lead to more sustained excessive alcohol consumption as demonstrated in monkeys.

The final model described involves linking procedures for inducing alcohol dependence with self-administration protocols. Most commonly, induction of dependence is accomplished by delivery of chronic alcohol exposure via the inhalation route. This route of administration has many advantages (e.g., ability to exert rigorous experimental control over variables such as duration, frequency, and intensity of exposure while minimizing compromised health), but a detraction relates to the fact that the inhalation procedure departs from the manner in which humans normally consume alcohol (orally). It is important to note, however, that models of dependence and relapse drinking are not designed to examine how dependence develops but, rather, the focus is on how a history of chronic alcohol exposure that renders subjects dependent alters motivational processes that engender excessive levels of consumption. As previously indicated, with few exceptions, rodents, even when given free access to alcohol, will not consume sufficient amounts to produce dependence. Thus, in order to study the impact of dependence on continued and sustained alcohol drinking, the dependence state must be experimentally induced. This approach has effectively been adopted in rat and mouse models, with stable alcohol intake first established and then followed by chronic alcohol exposure. In many cases, the chronic alcohol exposure is delivered in an intermittent rather than continuous fashion. Thus, these models incorporate alternating cycles of chronic alcohol exposure interspersed with periods when subjects have the opportunity to self-administer the drug. This relates to a positive feature of this model, namely that alcohol drinking can be evaluated in the context of both alcohol's positive and negative reinforcing effects. Indeed, the ability to contrast relatively stable alcohol consumption in nondependent subjects with dependent subjects that exhibit escalation of drinking is a powerful attribute of this approach that has been exploited in studies aimed at elucidating underlying neuroadaptations and motivational mechanisms as well as evaluation of pharmacological agents that influence alcohol consumption in the context of dependence.

In sum, a number of animal models have been developed to study excessive alcohol consumption in rodents. Each model possesses unique experimental characteristics that confer both advantages and disadvantages. No single approach can claim to capture all the complexities that define problem drinking in humans.

As in the case for scientific inquiry of all human disease states such as alcoholism, the selection of an animal model greatly depends on the specific research question under study. The five models described in this chapter may, individually, only reflect incomplete approximations of factors involved in excessive drinking behavior, but collectively, these models have been valuable as aids in advancing our knowledge about the biological and environmental contingencies that bear on this complex behavior. Despite these advances, however, challenges remain. In the continued quest to develop more optimal models, there is the need to incorporate procedures that more closely mimic variables that are most relevant to impacting drinking in humans. These include consideration of factors that underlie initial sensitivity as well as changes in perception and expectations regarding the link between intoxication and drinking as subjects gain more experience and exposure to the drug, cognitive (learning/memory) factors that guide decisions about engaging and terminating drinking behavior, and distinguishing circumstances in which environmental factors such as cues, stress, and timing and predictability of access exert different effects on propensity to drink. Ultimately, the validity and usefulness of these models will lie in their ability to serve as a platform for studying biological underpinnings and environmental influences that drive increased motivation for alcohol seeking and consumption, as well as evaluation of treatment strategies that effectively reduce excessive levels of alcohol consumption.

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Modeling Relapse Situations in the Human Laboratory

Rajita Sinha

Abstract It is well known that alcoholism is a chronic relapsing illness. While stress significantly impacts alcoholism risk, there is also evidence that increasing levels of alcohol use affect peripheral and central stress and reward pathways thereby setting up a reciprocal relationship among the effects of alcohol consumption of the development, course of and recovery from alcoholism. This chapter reviews our efforts in assessing the integrity of stress pathways in alcoholism by examining whether altered responses of the stress pathways play a role in relapse risk. Using validated human laboratory procedures to model two of the most common situations that contribute to relapse risk, we review how such models in the laboratory can predict subsequent alcohol relapse. Empirical findings from human laboratory and brain imaging studies are reviewed to show that specific stress-related dysregulation accompanies the alcohol craving state in alcohol-dependent individuals, and such dysregulation along with increases in alcohol seeking are predictive of increased alcohol relapse risk. Finally, the significant implications of these findings for the development of novel treatment interventions that target stress processes and alcohol craving to improve alcoholism relapse outcomes are discussed.

Keywords Relapse precipitants · Human laboratory models · Stress · Drug cues · Drug craving · Relapse risk

R. Sinha (✉)
Department of Psychiatry, Yale University School of Medicine,
34 Park Street Room S110, New Haven, CT 06519, USA
e-mail: rajita.sinha@yale.edu

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Contents

1 Introduction 380
2 The Challenge of Modeling Alcohol Relapse Risk in the Laboratory 381
2.1 Studying Stress, Cues and Alcohol Craving in the Laboratory 381
2.2 Chronic Alcohol-Related Changes in Emotion, Stress and Motivational Systems 382
2.3 Modeling Relapse Situations in the Laboratory 383
3 Developing a Valid Laboratory Model of Relapse Situations 383
3.1 Emotional Imagery Methods 384
3.2 Individualized Emotional Imagery Procedures 385
4 Provocation of Relapse Situations in the Laboratory 388
4.1 Provoked Alcohol and Drug Craving and Severity of Disease State 389
4.2 Stress Dysregulation and Enhanced Drug Craving in Addicted Individuals 391
5 Neural Correlates of Stress and Drug Craving in Addiction 392
6 Laboratory Response to Relapse Situations and Subsequent Alcohol Relapse 393
7 Specific Responses as Targets for Treatment Development 394
8 Implications for Treatment Development to Prevent Stress- and Cue-Related Relapse 395
9 Limitations and Caveats 396
10 Summary 396
References 397

1 Introduction

The last two decades have seen tremendous growth in neuroscience with significant advances in understanding the cellular and molecular correlates of addiction. Basic science research has identified novel molecular and cellular factors associated with addiction. Neuroadaptations in stress pathways and their interaction with reward and motivational circuits have been identified as critical in perpetuating the chronic relapsing nature of addictive disorders (Koob et al. 2004; Kreek and Koob 1998; Sinha 2001, 2007). With these advances in neuroscience, there is greater impetus to examine these mechanisms in humans and in the clinical context. This chapter describes the efforts of my laboratory in modeling real-world situations in the laboratory and assessing their contribution to alcohol relapse risk. Human laboratory studies have been used often to model drug effects, drug self-administration and desire, craving and urges for substances. In the human laboratory, our goal has been to provoke relapse risk situations and assess whether we can induce hallmark features of alcohol seeking and consumption and assess its subsequent effects on relapse susceptibility. An additional goal is to translate previously identified preclinical (animal and human) mechanisms of the alcohol disease state in the laboratory so as to provide a methodology for the development and testing of novel treatment interventions in humans.

2 The Challenge of Modeling Alcohol Relapse Risk in the Laboratory

2.1 Studying Stress, Cues and Alcohol Craving in the Laboratory

Environmental stimuli previously associated with drug use, or internal cues such as stress responses, negative affect and withdrawal-related states associated with alcohol and drug abuse, can function as conditioned stimuli capable of eliciting craving (Stewart et al. 1984) which can increase relapse risk. Classical conditioning is one mechanism by which neutral environmental cues paired with drug acquires emergent stimulus effects in contrast to stimuli paired with placebo drug (Foltin and Haney 2000; O'Brien et al. 1998). These data are consistent with many human laboratory studies documenting that exposure to external drug-related stimuli, which may include people and places associated with alcohol and drug use such as beer cans, bars, smoking cigarettes and drinking alcohol, seeing drinking buddies and other alcohol-related situations that involve drinking, can result in increased drug craving and physiological arousal related to the drug itself (Carter and Tiffany 1999a). Exposure to negative mood or withdrawal-related distress has also been associated with increases in drug craving and cue reactivity (Childress et al. 1994; Cooney et al. 1997) and our early laboratory studies showed increased alcohol and drug craving and arousal with exposure to personalized stress in the laboratory (Sinha et al. 2000; Sinha et al.1999; Sinha and O'Malley 1999). While interoceptive cues may become paired with drug effects and increase drug craving and physiological reactivity, the possibility that stress activation may directly affect craving and compulsive seeking and that conditioned emotional responses associated with drug cues may activate additional emotional motivational circuits that in turn affect craving and relapse processes was a possibility worth exploring in laboratory studies. Thus, laboratory studies were needed to understand the similarity and differences in different types of relapse situations and to examine how stress- and cue-related mechanisms may affect alcohol craving and relapse susceptibility in humans.

There is a growing literature that alcohol and drug abusing individuals show greater cue reactivity than recreational users of alcohol and drugs (Glautier et al. 1992; Greeley et al. 1993; Kaplan et al. 1985; Pomerleau et al. 1983; Willner et al. 1998). While social drinkers report increases in cue-induced alcohol craving, findings on behavioral and physiological responses to cues in social drinkers are weak and quite mixed in the literature (Carter and Tiffany 1999b; Litt and Cooney 1999). In other evidence, severity of alcohol use has been shown to affect the magnitude of cue reactivity, compulsive alcohol seeking and stress-related changes, including alcohol-related morbidity (Fox et al. 2005; Grusser et al. 2006, 2007; Rosenberg and Mazzola 2007; Sinha 2008a, b; Yoon et al. 2006). These data are consistent with large population-based studies indicating that with greater

amounts of weekly or daily alcohol and drug use, there is greater risk of alcohol-related problems, addiction and chronic diseases (Dawson et al. 2005; Rehm et al. 2009; Room et al. 2005). Thus, with increasing levels of alcohol and drug use, there appears to be greater craving responses. Whether such increases in craving and 'wanting' are mediated by neuroadaptations in stress and motivational systems that drive craving, compulsive seeking and drug use behaviors, notions that are consistent with recent incentive sensitization and allostasis models of addiction (Koob et al. 2004; Robinson and Berridge 1993), remains to be fully established in human studies. Human laboratory models provide a unique opportunity to test these hypotheses in humans and translate the understanding of the association between stress, craving and relapse susceptibility from basic science models of relapse into the clinical context.

2.2 Chronic Alcohol-Related Changes in Emotion, Stress and Motivational Systems

There is now solid evidence that regular and chronic alcohol use is associated with stress-related symptoms and changes in mental state which may include increased anxiety and negative emotions, changes in sleep and food intake, aggressive behaviors, alterations in attention, concentration and memory and desire/craving for alcohol (Sinha 2001, 2007). Stress-related symptoms are most prominent during early abstinence from chronic alcohol use, but some of these changes have also been documented during active use of specific drugs. Growing evidence from basic science studies and further corroboration from human neuroimaging indicate that chronic alcohol abuse alters reward and motivational responses, including alterations in dopaminergic activity, and that such changes are associated with increases in alcohol craving (Cleck and Blendy 2008; Gilman and Hommer 2008; Heinz et al. 2004, 2005; Koob and Kreek 2007; Koob et al. 2004; Martinez et al. 2007; Volkow 2004).

In other evidence, it has long been known that alcohol stimulates the hypothalamic-pituitary-adrenal (HPA) axis and initially stimulates the autonomic systems by provoking sympathetic arousal followed by depressing such activation (Ehrenreich et al. 1997; Lee and Rivier 1997). Dramatic adaptations of the HPA axis akin to tolerance has also been demonstrated with regular and chronic alcohol abuse in animals (Zhou et al. 2000; Richardson et al. 2008) and in humans (Adinoff et al. 1998, 2005; Wand and Dobs 1991). Similarly, chronic alcohol-related changes in autonomic responses, particularly in parasympathetic vagal tone has also been documented in non-human primates (Shively et al. 2007) and in humans (Ingjaldsson et al. 2003; Rechlin et al. 1996; Thayer et al. 2006). These data are consistent with changes in peripheral stress pathways which parallel other basic science findings of alcohol-related adaptations in the extrahypothalamic corticotrophin releasing factor (CRF) systems and the noradrenergic pathways that are consistent with an upregulated central CRF and noradrenergic pathways

(Rasmussen et al. 2006; Cleck and Blendy 2008; Koob and Kreek 2007; Koob 2009; also see Heilig et al. 2010 for review). These data document specific dysregulation in emotion, stress and motivational systems in alcoholics, and raise the question of whether these measures contribute to the high levels of emotional distress and the pathophysiology of alcohol craving and compulsive alcohol seeking associated with relapse susceptibility.

2.3 Modeling Relapse Situations in the Laboratory

There are many challenges to studying relapse situations and compulsive alcohol seeking in the laboratory. A key challenge is the ecological relevance of the provocation method, especially when studying psychopathological populations where the specific psychiatric illness is itself seen as a chronic distress state (Brady and Sinha 2005). For example there are widespread individual differences in relapse situations (McKay et al. 1995, 1996) and hence using experimental-derived standard provocateurs may not capture drug-related associations that are likely involved in craving- and relapse-related motivational processes. Another challenge is that as relapse situations often involve drug, drug-related, emotional or stressful and such stimuli invoke arousal of stress pathways, there is a need to address the well-known alterations in the 'normal' stress responses. Of course, one way to address these issues is by designing laboratory experiments with adequate within-group control conditions and/or between-group controls such as inclusion of non-addicted healthy controls or social drinkers. In our studies, we have increasingly added both in the experimental designs so that we can examine changes in motivational state as a function of exposure to relapse situations and changes in biological stress and arousal measures in comparing to healthy non-addicted individuals using comparable methods in the laboratory. Finally, an additional consideration is that of stress and craving measurement. Ensuring sensitivity in measurement of basal stress responses to detect adaptation pertaining to disease state could relate it to changes in motivation state and craving associated with provoked or challenge responses.

3 Developing a Valid Laboratory Model of Relapse Situations

In order to develop a validated method to study relapse situations in alcoholics and drug abusers in the laboratory, the method needs to achieve four objectives as outlined in our previous review (Sinha 2009). The method should (a) consistently reproduce a hallmark disease symptom, such as craving, in the laboratory setting, thereby providing internal validity; (b) provoke the particular disease symptom which in turn, should be associated with alcoholism severity; (c) be predictive of alcohol use behaviors and real-world clinical outcomes; and finally (d) be responsive to interventions, i.e., making the disease worse or better.

In the clinical context, alcoholic patients entering outpatient substance abuse treatment report high levels of stress and an inability to manage distress adaptively, thereby increasing the risk of succumbing to high levels of drug craving and relapse to drug use (Sinha 2007). While patients are often successful in learning cognitive-behavioral strategies in the clinic, relapse rates remain high (Brandon et al. 2007), suggesting difficulties in applying and accessing these strategies in real-world relapse situations. The focus of our laboratory studies became the development of an ecologically relevant method that models such relapse risk in real-world situations in order to understand the biobehavioral mechanisms underlying relapse susceptibility. One key feature of our method was to provoke two of the most common relapse situations, namely emotionally stressful situations and drug-related situations in order to develop a comparable method of provoking stress- and the drug-related craving state. A second key aspect was to build in an experiment control condition to account for the non-specific aspects of the experimental procedures.

3.1 Emotional Imagery Methods

Emotional imagery paradigms have been widely used in behavioral and neuroimaging research to understand the pathophysiology of mood and anxiety disorders, including major depression, panic disorder, obsessive compulsive disorder and post-traumatic stress disorder (Cook et al. 1988; Foa and Kozak 1986; Mayberg et al. 1999; McNeil et al. 1993; Orr et al. 1993, 1998; Pitman et al. 1987; Shalev et al. 1993; Teasdale et al. 1995). They have also been used for anger provocation to assess anger effects on markers of cardiovascular disease (Nelson et al. 2005). There is also a body of research using imagery procedures to study the effects of affect and cues on nicotine craving in the laboratory (Cepeda-Benito and Tiffany 1996; Drobes and Tiffany 1997; Maude-Griffin and Tiffany 1996; Tiffany and Drobes 1990; Tiffany and Hakenewerth 1991). While the mood and anxiety disorders literature had moved to the use of individualized script scenarios, the work of Tiffany and colleagues was primarily based on standard and generic scripts for induction of nicotine cue reactivity.

The emotional imagery method has been developed by Lang and colleagues based on the premise that emotional imagery activates the same physiological, subjective and behavioral responses as emotions in real life, thus being a potent, ecologically valid research procedure to study emotional experiences. According to Lang (1977, 1979), emotions are represented as networks in memory and include three kinds of information: (a) information about the specific stimulus context, (b) information about verbal, physiological and overt behavioral responses, and (c) interpretive information about the meaning of the stimulus and response elements of the structure. Activation of any network "node" or component would activate the full network and produce the emotional experience in question. In developing and validating this method in studies on the

psychophysiology of fear and anxiety, Lang found that the more the number of stimulus aspects as well as physiological, behavioral and emotion consistent cognitive responses that were included into the imagery induction script, the stronger the activation produced by the imagery procedure (Lang et al. 1980, 1983). He and his colleagues also found that when the imagery scripts were based on *personal* fear scenarios compared to standard fear, anger and anxiety scripts, subjects showed stronger physiological and subjective responses (Cook et al. 1988; McNeil et al. 1993; Miller et al. 1987). In our earlier studies on the psychophysiology of emotions, we reported significant physiological responses associated with specific emotion states in healthy volunteers, using individualized scripts for the primary emotions of fear, sadness, anger, fear, joy and neutral-relaxed states (Sinha et al. 1992; Sinha and Parsons 1996). On the basis of this previous theoretical and empirical knowledge on provocation of emotions, we developed a standardized method to elicit individualized real-world relapse situations from subjects that involved emotional stress, drug-related scenarios and a neutral-relaxing scenario as a control situation.

3.2 Individualized Emotional Imagery Procedures

Individualized guided imagery procedures involve an initial imagery script development session, standardized script generation and auditotaping, followed by a habituation and imagery training session that precedes the experimental sessions [full description of procedures is provided in Sinha (2001 Manual for imagery script development procedures. Unpublished manuscript) and Sinha and Tuit (2011)]. The experimental method involves development of scripts for stress, emotions and/or alcohol-related stimuli along with a non-specific control script, each based on the subject's individual experiences. Below is a sample script development session, lasting approximately 1 h, which involves developing a single script from a stressful situation, an alcohol-related craving and consumption situation and a neutral-relaxing situation. The conditions are presented in random order and counterbalanced across subjects. Subjects remain blind to the order and type of condition until the presentation of audiotape, while the experimenters remain blind to the order and content of each audiotape during laboratory sessions.

3.2.1 Imagery Script Development Session

In a session prior to the laboratory sessions, scripts for the guided imagery induction are developed. The *stress imagery script* is based on subjects' description of a recent event that the subjects experienced as "most stressful". Stress is defined for each subject as a situation that made them "sad, mad or upset and in that moment they were not able to change the situation". Subjects individually calibrate the situation by rating their perceived stress experienced in that particular

situation on a 10-point Likert scale where "1 = not at all stressful" and "10 = the most stress they felt recently in their life". Only situations rated by the subjects as 8 or above on this scale are accepted as appropriate for script development. This procedure ensures that each stress script is individually calibrated for the level of subjective stress across subjects. Traumatic situations or stressful situations that involved drug-related stimuli, such as being arrested for possession of drugs or being caught in a police chase, are not allowed. Examples of acceptable stressful situations include breakup with significant other, a verbal argument with a significant other or family member or unemployment-related stress, such as being fired or laid off from work.

The *alcohol-related script* is developed by having subjects identify a recent situation that included alcohol-related stimuli and resulted in subsequent alcohol use (e.g., buying alcohol, being at a bar, watching others drink alcohol; getting together with alcohol-using drinking buddies). Alcohol-related situations that occurred in the context of negative affect or psychological distress are not allowed, i.e., going to a bar after a marital conflict, or feeling depressed and calling a drinking buddy. A *neutral-relaxing script* is developed from the subjects' commonly experienced neutral-relaxing situations. Neutral-relaxing events that involve drugs, people associated with drugs or those involving high arousal are not allowed.

A 'script' or description of each situation (script length varies based on the aim of the study and the associated methodological issues), is developed using Scene Development Questionnaires [SCQ, adapted from Lang et al. (1980), presented in Sinha R (2001 Manual for imagery script development procedures. Unpublished manuscript)] which obtain specific stimulus and response details, including specific physical and interpersonal context details, verbal/cognitive attributions regarding the situation, and physiological and bodily sensations experienced for the situation being described. While the scripts include individual context information and are therefore personalized, they have standard style that is replicated across all scripts. Table 1 presents sample scripts for each condition from alcohol-dependent individuals. The three scripts for each subject are then recorded on an audio-tape as stimuli for guided imagery in the experimental sessions.

3.2.2 Manipulation Check for Script Development

All three scripts are also rated on a Likert scale from 1 to 5 on a standard rating form (Independent Rating Scale) by two objective independent raters for stressful and emotional content. If a stress imagery script scores below a rating of 3 for stressful content on a 5-point rating scale the subject will be asked to develop a new script at the next appointment prior to the laboratory sessions. On the other hand, if the alcohol-related script scores above a "3" for stressful or emotional content, the subject will develop a new alcohol-related script at the next appointment. These procedures ensure that the stress- and alcohol-related scripts are equated in intensity and content. It further ensures that differences in stress reactivity are not due to differences in intensity and emotional content of the stressor.

Table 1 Sample individualized scripts

Stress	It is Saturday night about 5:00 p.m. R_ picks you up at your apartment and you are on your way to the bowling alley. As you get in the car, you ask her, "Do you love me or not?" She wants to talk to you about this tonight. Your heart beats faster. She drives the car and you press her to tell you what's on her mind. There is silence in the car. You feel tense all over your body. You are not sure what's going on, but it doesn't sound good. She stops the car in the parking lot. Your face is tight. Gritting your teeth, you ask her to tell you right now. She starts by saying, "we are always fighting, and I really need to focus on school". Your stomach is in a knot. She blames you for acting too much like her father. You feel hot all over. You can't believe she is saying this, and you dread what's coming next. She doesn't want to see you any more! You feel choked up. You try to explain to her you have always tried to help and be there for her. She says she doesn't like you when you get moody, loud and upset at her. Blood rushes to your head. How come she never said anything before. You start to wonder if she has been seeing other people. Your jaws are clenched. You want to scream and strike someone. You feel betrayed and alone. You want to say so much but no words come out. Tears come to your eyes and you just want to go home.
Drug-related script	It is the morning of July 4. You are at the Lighthouse Beach for the first picnic of the summer. Your eyes glance up at the cloudless blue sky. You see the barbecues all set up. You feel a sense of being more alive. You watch the flames are burning high. You feel pumped up. There are coolers spread out all over the beach. There are over 50 people here already, and the party is finally getting started. Almost everyone has a drink in their hands already. There are butterflies in your stomach. Now you really want a drink. You want to get all nice. You eyes glance over to one of the coolers. You flip the lid and take a look inside. Your heart beats faster. You see bottles of Beer, Gin, Scotch, Vodka, and Rum. You are breathing faster. You reach into one of the coolers and bury your hand in the ice. Your hand feels cold and wet. There is a tingling sensation inside you. You grab onto a can of beer and pull out your arm. You give it a shake to remove some of the excess water. You wet your lips as you crack it open. You raise the beer to your mouth and tilt it back. It feels ice cold against your lips. There is a rush of excitement inside you. You let the beer flow into your mouth and slide down your throat. There is a warmth inside you. Now you have a taste for it. You are ready for another big swig.
Neutral-relaxing script	You are sitting on the beach on a bright summer day. You breath in deeply as you notice a red kite against the cloudless blue sky. Your eyes trace the path of the kite as it whips up and down in spirals with the wind. The sun glares at you from behind the kite and makes the white sandy beach sparkle with reflection. You tense the muscles in your forehead and around your eyes, squinting to block out the bright sunlight. You follow with your eyes the long white tail, which dances from side to side beneath the soaring kite. You take in a few deep breaths of the fresh ocean air, noticing the smells of the fish and the salt water. The warm sun beats down against your skin and a light gentle breeze blows over you. You listen to the soothing sound of the ocean waves, roaring and splashing as it comes onto the sand, and quiet as the water goes back out to sea. You relax the muscles in your arms, back and legs as you lay back on the sand, feeling the soft fine granules of sand between your toes and fingers. The tension from your body goes down and you feel comfortable and at ease. Your breathing slows down and the worry thoughts seem to fade away. There is a sense of lightness and you want to hold time and capture this moment. A feeling of peace overcomes you.

3.2.3 Imagery and Relaxation Training

Although all individuals are able to imagine situations especially from their own lives, imagery ability varies across individuals and it has been found that imagery and relaxation training increase the emotional responses during imagery provocation (Miller et al. 1987). Thus, subjects are provided with relaxation training followed by general imagery and physiological response training (fully described in the imagery training procedures manual; Sinha, 2001 Manual for imagery script development procedures. Unpublished manuscript). The imagery training involves subject visualizing some commonplace scenes as they are presented to them. The scenes are neutral and non-emotional in content, such as reading a popular magazine. Following the imagery, the subject is asked questions about the visualization and given pointers regarding the process of imagining the scene. The subject also imagines scenes that are non-emotional but physically arousing in nature, such as doing sit-ups in gym class. With these scenes subjects are asked whether they notice any changes in their physiological response, such as change in heart rate or change in breathing. Once again, pointers in regard to imagining the situation “as if” they were really present in the situation are presented. The relaxation and imagery training procedure takes approximately 1 h and was developed to ensure that all subjects are trained on the method of generating an image and maintaining it for 2–3 min.

4 Provocation of Relapse Situations in the Laboratory

In developing a validated laboratory model for relapse situations, we targeted alcohol and drug craving as a primary outcome measure that is both a common feature of alcoholism and substance abuse and is also known to relate to the disease state. In our initial studies, we compared a commonly used standard social stress task, giving a speech in front of a video camera with the potential for a monetary reward, and compared that method to 5-min individualized guided imagery exposure of subjects’ own recent stressful scenarios. We found that in addicted individuals, stress imagery elicited multiple emotions of fear, sadness and anger as compared to the stress of public speaking, which elicited increases in fear but no anger and sadness. In addition, individualized stress imagery resulted in significant increases in drug craving while public speaking did not (Sinha and O’Malley 1999). In the next study, we examined stress-induced and drug-related craving and physiological responses using individualized scripts of comparable length and style for stress-, drug- and neutral-related situations. Significant increases in heart rate, salivary cortisol levels, drug craving and subjective anxiety were observed with imagery exposure to stress and non-stress drug cues as compared to neutral-relaxing cues in cocaine-dependent individuals (Sinha et al. 2000). Using these methods, we have been able to reliably induce alcohol and drug craving in multiple groups of treatment engaged cocaine-, alcohol- and

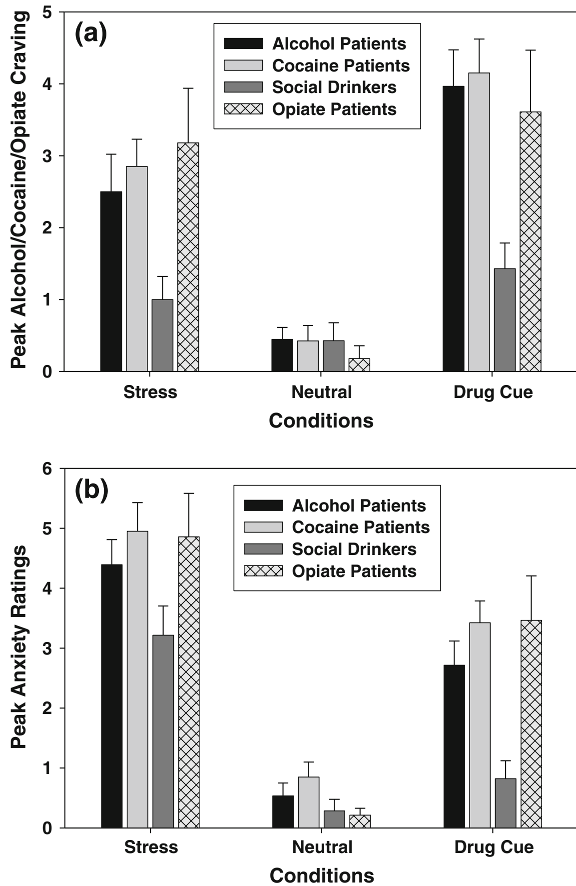

Fig. 1 Mean and standard errors for peak drug craving and anxiety ratings during exposure to stress, drug cues and neutral imagery conditions in separate groups of alcoholic-, cocaine- and opioid-dependent individuals and healthy social drinkers combined across three studies (Fox et al. 2008; Hyman et al. 2007; Sinha 2008a, b). **a** Peak craving during stress and drug cue conditions is significantly higher in abstinent alcoholics-, cocaine- and naltrexone-treated opioid patients compared to social drinkers ($P < 0.0001$ for both conditions). **b** Peak anxiety ratings is significantly higher in abstinent alcoholics-, cocaine- and naltrexone-treated opioid patients compared to social drinkers in both stress and drug cue conditions (stress: $P < 0.005$; drug cue: $P < 0.0001$)

opiate-dependent individuals and increase desire for drug in healthy social drinkers (see Fig. 1) (Chaplin et al. 2008; Fox et al. 2007; Hyman et al. 2007; Sinha et al. 2003). In addition, mild to moderate levels of physiological arousal and subjective levels of distress were found to accompany the alcohol/drug craving state (see Fig. 2).

4.1 Provoked Alcohol and Drug Craving and Severity of Disease State

In the second criteria for validated models of relapse situations, it is specified that the key outcome measure associated with alcoholism disease state should vary as a function of severity of disease state. We hypothesized that if drug craving and associated stress dysregulation are indeed factors affected by chronic alcohol and

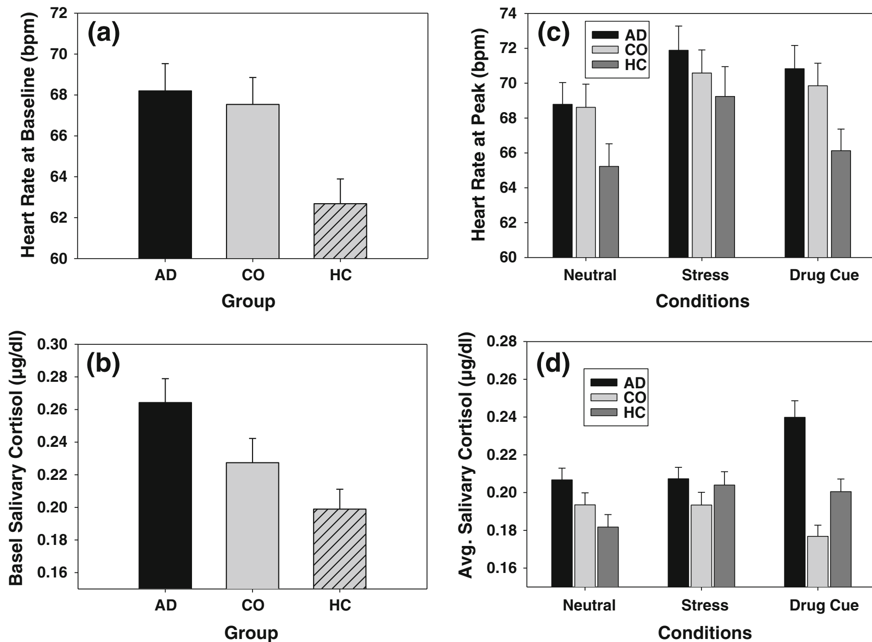

Fig. 2 Mean and standard errors for: **a** basal heart rate measured at baseline prior to imagery exposure over several time points across three separate days of experimental testing in alcoholic (AD) and cocaine (COC) patients compared to social drinkers (SD) (AD > COC > SD, $P < 0.01$); and **b** basal salivary cortisol averaged for two baseline assessments across three separate testing days (AD > SD, $p < 0.01$; COC > SD, $P < .11$); **c** peak heart rate responses in the stress (S), drug cue (D) and neutral (N) conditions: SD group shows S > N, $P < 0.0001$; D > N, $P < 0.02$; S > D, $P < 0.04$), while AD group only shows increases response in D > N, $P < 0.01$; and no differences between conditions in the COC group; **d** average salivary cortisol responses to stress (S), drug cue (D) and neutral (N) conditions: SD group shows S > N, $P < 0.05$, AD group shows D > N, $P < 0.05$, and COC group shows no differences between groups in responses [combined data averaged from Fox et al. (2008) and Sinha (2008a, b)]

drug abuse, then severity of alcohol and drug abuse should affect craving and stress dysregulation. We examined severity of cocaine and alcohol use among addicted individuals by dividing the cocaine and alcohol addicted sample (all of whom met dependence criteria) into those who were using alcohol and drugs at a high frequency, i.e., greater than 3 times per week, prior to inpatient admission for research, versus those who used drugs at a lower frequency of less than 3 times per week and assessed whether individuals with higher severity of drug abuse showed greater drug craving, anxiety and more stress dysregulation. Findings indicated that addicted individuals using cocaine and alcohol four or more days per week showed greater drug craving, anxiety and associated cardiovascular and HPA response to both stress and alcohol/drug-cue exposure as compared to those using 3 days or less per week (Fox et al. 2005). Thus, consistent with epidemiological

and clinical studies cited earlier, the emotional imagery-based laboratory method was found to be sensitive to severity of disease state in both drug craving and associated anxiety and in level of stress dysregulation.

4.2 Stress Dysregulation and Enhanced Drug Craving in Addicted Individuals

In the previous sections, we summarized the findings where laboratory studies reliably induced stress- and cue-induced alcohol and drug craving in multiple groups of addicted samples, and with evidence of stress-related physiological changes with stress and with drug cue exposure as compared to neutral imagery exposure. Initial evidence of the effects of disease severity on these responses was also observed. We also investigated whether these responses are altered or dysregulated in early abstinent alcoholics in comparison to non-addicted, social drinking controls. One potential drawback of standard laboratory stress provocation tools has been that patients may not find methods such as a public speaking or a math problem meaningful and relevant to their lives which could differentially affect participation in the stress provocation between addicted samples and controls. In contrast, individualized emotional imagery procedures account for potential differential effects of stress provocation by individually calibrating the level of stressfulness among subjects. Thus, there are no differences between controls and patients on stressfulness ratings of their stress scenarios. Comparability across alcohol-related scenarios is not problematic as these situations are elicited for presence of alcohol and drug-related stimuli, leading to wanting alcohol and subsequent alcohol use itself.

We compared 4-week abstinent alcoholics to matched social drinkers (drinking less than 25 drinks per month). The recovering alcoholics at 4-week abstinence showed greater levels of basal heart rate and salivary cortisol levels compared to control drinkers. Upon stress and alcohol cue exposure, they showed greater subjective distress, alcohol craving and blood pressure responses, but a blunted stress-induced heart rate and cortisol responses compared to controls (Sinha et al. 2009). Furthermore, alcoholic patients showed a persistent increase in alcohol craving, subjective distress and blood pressure responses across multiple time-points as compared to social drinkers, suggesting an inability to regulate the high alcohol craving and emotional stress state. These data indicate greater allostatic load in abstinent alcoholics accompanied by dysregulated stress responses and high levels of craving or compulsive seeking for the preferred drug.

Together, these data indicate that stress responses are altered in alcoholics and these alterations also include an enhanced susceptibility to stress- and cue-induced alcohol seeking which is not seen in healthy non-addicted individuals (see Fig. 1). Furthermore, there are basal alterations in peripheral markers of stress, indicative of stress-related dysregulation in the CRF-HPA axis and in autonomic responses as measured by basal salivary cortisol and heart rate responses; these high basal

responses are associated with lower or blunted stress-related arousal, similar to other high and chronic distress states (Li et al. 2007; Sinha et al. 2000; Steptoe and Ussher 2006). It is important to note that these alterations were not accounted for by the smoking status or lifetime history of anxiety or mood disorders and therefore appear to be related to the history of chronic alcohol abuse. The persistence of emotional distress and alcohol craving induced by stress and alcohol cue exposure suggests a dysfunction in emotion regulatory mechanisms. As HPA axis responses and autonomic-parasympathetic responses contribute to regulating and normalizing stress responses and regaining homeostasis, dysfunction in these pathways and their related central mechanisms may be involved in perpetuating alcohol craving and relapse susceptibility.

5 Neural Correlates of Stress and Drug Craving in Addiction

With the emergence of functional neuroimaging technology in the last 15 years, effective experimental methods to assess drug craving, emotions and stress within the confines of neuroimaging procedures have been developed. Using a variety of cue induction procedures, many studies have examined brain regions associated with craving in addicted individuals. Exposure to drug cues is known to increase craving increases activity in the amygdala and regions of the frontal cortex (Childress et al. 1999; Grant et al. 1996; Kilts et al. 2001). Gender differences have also been reported in cue-related activation in the amygdala and frontal cortex in cocaine-dependent individuals (Kilts et al. 2004; Li et al. 2005). Cue-induced craving for nicotine, methamphetamine and opiates also activate regions of the prefrontal cortex, amygdala, hippocampus, insula and the Ventral Tegmental Area (VTA) (see Sinha 2007). Having successfully modeled stress-induced craving experimentally in the laboratory, we also examined brain activation during stress and neutral imagery in a functional magnetic resonance imaging (fMRI) study. Although healthy controls and cocaine-dependent individuals showed similar levels of distress and pulse changes during stress exposure, brain response to emotional stress in paralimbic regions such as the anterior cingulate cortex, hippocampus and parahippocampal regions was observed in healthy controls during stress while cocaine patients showed a striking absence of such activation (Sinha et al. 2005). In contrast, patients had increased activity in the caudate and dorsal striatum region during stress, activation that was significantly associated with stress-induced cocaine craving ratings. Similarly, stress, alcohol cue and neutral imagery exposure was assessed in social drinkers and robust and similar activation of corticolimbic striatal regions were seen with stress and alcohol cue exposure. Alcohol cue-induced ventral and dorsal striatal activity correlated with alcohol cue-induced craving in men (Seo et al. 2010a).

Recent studies using Positron Emission Tomography (PET) have also shown significant positive correlations between the dorsal striatum and drug cue-induced cocaine craving (Volkow et al. 2006; Wong et al. 2006). These findings are

consistent with imaging studies with alcoholic patients showing increased association between dorsal striatum regions and alcohol craving in response to presentation of alcohol-related stimuli (Grusser et al. 2004; Wrase et al. 2002). Using PET imaging with alcoholics and cocaine patients, research has shown a significant association between dopamine D2 receptor binding in the VS and drug craving as well as motivation for self-administration (Heinz et al. 2004; Martinez et al. 2005, 2007). On the other hand, neuropsychological and imaging studies examining prefrontal executive functions, including impulse control, decision making and set shifting, have shown executive function deficits and hypo-frontal responses in addicted individuals compared to control volunteers (Ersche et al. 2005, 2006, 2008; Hester and Garavan 2004; Kaufman et al. 2003; Li and Sinha 2008; Noel et al. 2007; Paulus et al. 2006). Together, these data show a distinct pattern of findings indicating that increased stress- and cue-induced craving and compulsive drug- seeking states in addicted individuals are associated with greater activity in the striatum, but decreased activity in specific regions of the cingulate and prefrontal cortex and related regions involved in controlling impulses and emotions (Li and Sinha 2008).

6 Laboratory Response to Relapse Situations and Subsequent Alcohol Relapse

An important aspect of modeling hallmark addictive symptoms such as alcohol craving in the laboratory is to understand its related mechanisms and also to demonstrate the validity of the model by examining whether it shows predictive power with regard to actual drug use behaviors and/or real-world clinical outcomes. Because our laboratory studies described earlier were conducted with treatment engaged alcoholics and drug abusing samples who were inpatients at a treatment research unit, we were able to add a careful assessment of relapse once patients were discharged. This allowed us to examine specific markers of the stress and craving states that are predictive of relapse outcomes. Thus we followed inpatient treatment engaged alcohol-dependent individuals in our studies after discharge following completion of 5 weeks of inpatient alcohol treatment, for 90 days to assess relapse outcomes. Face-to-face follow-up assessments were conducted at 14, 30, 90 and 180 days after discharge from the inpatient unit. Our follow-up rates for these assessments have been 96, 89, 92, and 86% respectively.

Our initial evidence from assessing whether laboratory responses to stress- and alcohol-related stimuli exposure are predictive of alcohol treatment outcomes were positive. We found that stress-induced alcohol craving in the laboratory during inpatient treatment was predictive of number of days of alcohol used and total number of drinks consumed during the 90-day follow-up period (Breese et al. 2005). These data corroborate our findings in cocaine abusers showing that stress-induced cocaine craving and HPA arousal are associated with earlier relapse and more cocaine use at follow-up (Sinha et al. 2006). More recent data indicate that

both stress and alcohol cue-induced craving are associated with time to alcohol relapse (Sinha et al. 2011). Furthermore, blunted or low levels of stress-induced ACTH and heart rate responses, but higher cortisol/ACTH ratio at baseline and for stress and neutral condition are each predictive of shorter times to relapse (Sinha 2008a, b; Sinha et al. 2011). These data are consistent with some earlier reports of stress system involvement in relapse outcomes in alcoholics. Negative mood and stress-induced alcohol craving and blunted stress and cue-induced cortisol responses have been associated with alcohol relapse outcomes (Breese et al. 2005; Cooney et al. 1997; Junghanns et al. 2003). Thus, for alcoholic samples, as in the cocaine group, it appears that the drug craving state marked by increasing distress and compulsive motivation for drug (craving) along with poor stress regulatory responses (altered HPA responses or increased noradrenergic arousal) results in an enhanced susceptibility to addiction relapse.

Findings from our neuroimaging study that modeled stress and alcohol cue exposure in a functional MRI study, found hyper-responsivity of the ventromedial prefrontal cortex (VmPFC) and ventral striatum during neutral relaxed imagery and blunted responses of these regions during stress and alcohol cue exposure (Seo et al. 2010b). Higher activation of the VmPFC during the neutral condition and blunted response during stress and alcohol cues significantly predicted the level of stress-induced and alcohol cue-induced craving and concomitant anxiety during stress and alcohol cue exposure. Both hyper-responsivity of the ventral striatum and VmPFC during neutral relaxed states and blunted response during stress states were associated with a greater propensity to relapse (Seo et al. 2010b). Normalization of these regions that are critical in integration of emotional-motivational function would therefore be an important target of recovery and treatment of alcoholism.

7 Specific Responses as Targets for Treatment Development

The previous sections describe our approach to modeling relapse situations in the laboratory in alcoholics and summarized the evidence thus far, on whether stress and alcohol cue- related craving and stress dysregulation are predictive of alcohol relapse outcomes. The findings indicate that both stress and alcohol cue-induced provoked craving could serve as markers of relapse susceptibility. Altered HPA activity, especially cortisol/ACTH ratio as a marker of adrenal sensitivity was also found to be associated with relapse. Among brain imaging responses, altered stress-related activity in the VmPFC was also a sensitive measure of relapse. While anxiety and negative emotions such as anger, fear and sadness ratings were not predictive of relapse, they were significantly correlated with stress- and cue-induced alcohol craving and may serve as secondary target measures, which in conjunction with alcohol craving, could provide useful indicators of change in emotional distress associated with alcohol craving. All of these could serve as outcome measures for

further experimental testing of novel pharmacological and behavioral treatment interventions to prevent stress- and cue-related alcohol relapse.

8 Implications for Treatment Development to Prevent Stress- and Cue-Related Relapse

Having validated a human laboratory model with effective provocation methods and reliable measures of stress and drug craving, we have recently begun to test novel pharmacological agents that may decrease stress- and cue-induced alcohol craving and normalize stress regulation using agents that have shown promise in basic science models and/or in the clinical setting. One of the key advantages of using human laboratory models for this purpose is that they provide a cost-effective and efficient way to assess new approaches prior to undertaking large-scale clinical trials.

Several animal models of relapse have shown that overactive brain CRF, noradrenergic and glutamatergic systems along with underactive dopamine and GABA systems contribute to the high craving states and the chronic relapsing nature of addiction (Goeders 2002; Kalivas and Volkow 2005; Koob et al. 2004; O'Brien 2005; Shaham et al. 2003; Vocci et al. 2005; Weiss 2005). For example, using animal models of drug self-administration and reinstatement, preclinical studies have shown CRF antagonists and α-2-adrenergic agonists to be efficacious in reducing stress-related drug seeking in addicted laboratory animals (see Shaham et al. 2003; Weiss 2005 for review). Similarly, α1-adrenergic antagonists such as Prazosin have been found to decrease alcohol withdrawal symptoms, alcohol consumption and stress-induced relapse in animal models (Gilpin et al. 2009; Rasmussen et al. 2006; Walker et al. 2008) and in a pilot clinical study of alcoholics (Simpson et al. 2009).

We have previously tested whether the α-2-adrenergic agonist, lofexidine, is effective in decreasing emotional stress, physiological arousal and stress-induced drug craving in opiate-dependent individuals in naltrexone treatment. Although naltrexone, an opiate antagonist, is approved in the treatment of opioid addiction, it is not used widely because of poor compliance and high relapse rates. Thus, it provided a good model to conduct proof of concept studies to assess whether stress-related relapse can be decreased with lofexidine. In an initial small laboratory study, we demonstrated that naltrexone-treated opioid-dependent individuals showed high levels of stress- and cue-induced drug craving, physiological arousal and emotional distress when compared to neutral relaxing stimuli (Hyman et al. 2007). In a second study, lofexidine was found to significantly decrease stress-induced opiate craving and anger ratings while also decreasing basal heart rates and improving opiate relapse outcomes in a small study of naltrexone-treated opiate-dependent individuals (Sinha et al. 2007). Most recently in treatment engaged alcoholics, we have found that stress and alcohol cue-induced alcohol

craving, anxiety and stress dysregulation were each decreased relative to neutral responses with Prazosin and not in placebo-treated alcoholics (Fox et al. 2011). These studies provide initial support for the use of the human laboratory model to study relapse risk especially as a tool for testing novel pharmacological interventions.

9 Limitations and Caveats

It is important to acknowledge that modeling relapse situations in laboratory settings remain a challenge in the field. Several caveats about the emotional imagery methods and procedures used to provoke relapse situations in the laboratory need to be highlighted. First, while the emotional imagery method is effective and has been used in the study of mood and anxiety disorders and in cardiovascular disease, our adaptation of these methods is manualized, technically rigorous and therefore time-consuming and resource intensive. For example, individual script development sessions are conducted and then scripts for each condition per subject are developed. Only highly trained research staff who have completed a structured script development training and are certified for script development should be developing such stimuli for laboaratory situations. Our procedures have been tested in treatment-engaged addicted patients and healthy individuals and hence its effectiveness in non-treatment seeking and actively using individuals are not known.

While this review described the specific methods used in our laboratory studies, there are other experimental factors that are important to consider in studying relapse situations. For example, duration of the exposure to relapse situations may significantly affect strength of response. For example, in the laboratory studies, our script length is 5–6 min while in the brain imaging session they are 2 min long. Furthermore, in a medication study currently underway, we are using 10-min exposure periods with two scripts for each condition. Other factors, such as aversiveness of the relapse situation, its intensity and controllability are all factors that impact laboratory responses and ultimately affect the ability to detect individual differences in the laboratory, especially in clinical samples, with respect to clinical outcomes.

10 Summary

This paper describes the development and validation of a human laboratory model to assess chronic alcohol-related neuroadaptations in abstinent alcoholics. As alcohol-related neuroadaptations specifically affect the stress and reward pathways, the particular challenge of studying the most common relapse triggers, such as, stress and alcohol cues and associated alcohol craving is discussed.

Four criteria for development of a valid human laboratory model for alcohol-related adaptations and assessing relapse risk is outlined. Evidence from human laboratory and neuroimaging studies that show specific neuroadaptive changes in stress pathways and whether such changes alter subjective affect, alcohol craving and relapse risk is presented. Specific responses that are predictive of alcohol relapse risk are identified, and their use to screen novel pharmacological agents that show promise in reducing stress and cue-induced alcohol craving and normalizing stress dysregulation is discussed. Availability of such valid human laboratory models provides an important step towards development of new treatment targets to decrease alcohol relapse risk and improve clinical outcomes in the future.

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Modeling Relapse in Animals

Rémi Martin-Fardon and Friedbert Weiss

Abstract Alcohol addiction is a chronically relapsing disorder characterized by compulsive alcohol seeking and use. Alcohol craving and long-lasting vulnerability to relapse present a great challenge for the successful treatment of alcohol addiction. Therefore, relapse prevention has emerged as a critically important area of research, with the need for effective and valid animal models of relapse. This chapter provides an overview of the repertoire of animal models of craving and relapse presently available and employed in alcoholism research. These models include conditioned reinstatement, stress-induced reinstatement, ethanol priming-induced reinstatement, conditioned place preference, Pavlovian spontaneous recovery, the alcohol deprivation effect, and seeking-taking chained schedules. Thus, a wide array of animal models is available that permit investigation of behaviors directed at obtaining access to alcohol, as well as neurobehavioral mechanisms and genetic factors that regulate these behaviors. These models also are instrumental for identifying pharmacological treatment targets and as tools for evaluating the efficacy of potential medications for the prevention of alcohol craving and relapse.

Keywords Conditioned reinstatement · Conditioned place preference · Alcohol deprivation effect · Reinstatement · Pavlovian spontaneous recovery · Alcohol-seeking behavior

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R. Martin-Fardon · F. Weiss (✉)
Molecular and Integrative Neurosciences Department,
The Scripps Research Institute, La Jolla, CA 92037, USA
e-mail: bweiss@scripps.edu

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Contents

1 Introduction 404
1.1 Models of Craving and Relapse Associated with Conditioning Factors 405
2 Conditioned Reinstatement 405
2.1 Discrete Cues 408
2.2 Contextual Cues 408
2.3 Neurocircuitry of Conditioned Reinstatement 409
3 Pavlovian Spontaneous Recovery 410
4 The Operant Runway Model of Relapse 411
5 Conditioned Place Preference Models 411
5.1 Expression of Conditioned Place Preference as a Model of Relapse 412
5.2 Reactivation of Conditioned Place Preference 413
6 Modeling Craving Induced by "Priming" Doses of Ethanol 413
7 Seeking-Taking Chained Schedules 414
7.1 Dissociation of Alcohol-Motivated Appetitive and Consumatory Behavior 414
7.2 Chained Schedules as Potential Measures of Compulsive Alcohol Seeking 415
8 Models of Stress as a Risk Factor for Relapse 415
8.1 Footshock Stress 416
8.2 Pharmacological Stressors 416
8.3 Interactive Effects Between Stress and Conditioning Factors 417
9 The Alcohol Deprivation Effect (ADE) as a Relapse Model 419
10 Conclusions 420
References 422

1 Introduction

Alcoholism is a chronically relapsing condition characterized by compulsive drug seeking and use (American Psychiatric Association 2000; McLellan et al. 2000; O'Brien et al. 1998; O'Brien and McLellan 1996). Three factors have been implicated in vulnerability to relapse. These include learned responses evoked by environmental stimuli that have become associated with the subjective actions of drugs of abuse by means of classical conditioning. Exposure to such stimuli evokes drug desire and drug seeking effects that have been implicated both in maintaining ongoing alcohol and drug use and eliciting drug seeking and relapse during abstinence (O'Brien et al. 1996, 1998). A second factor with an established role in relapse to alcohol use in humans is stress. Not only is stress a precipitating factor for alcohol seeking, but chronic alcohol use and withdrawal elicit stress-like states, and withdrawal-related distress is associated with increased drug craving and conditioned cue reactivity, thereby compounding relapse risk associated with alcohol cue exposure (e.g., Brown et al. 1995; Kreek and Koob 1998; Marlatt 1985; McKay et al. 1995; Sinha 2000, 2001; Sinha et al. 1999, 2006). A third major factor in vulnerability to relapse is neuroadaptive dysregulation induced by chronic alcohol use (Koob 2003; Koob and Le Moal 2008). Such disturbances are thought to underlie symptoms of anxiety, mood disturbances, irritability,

autonomic arousal, and exaggerated responsiveness to stress that emerge when drug use is discontinued and outlast physical withdrawal and detoxification (e.g., Brower and Perron 2010; Heilig et al. 2010; Majchrowicz 1989; Martinotti et al. 2008; Meyer 1996).

To establish the neurobiological mechanisms that mediate ethanol-seeking behavior associated with these risk factors and to identify pharmacotherapeutic targets for relapse prevention, animal models are indispensable. This chapter provides an overview of animal models that are most widely employed in contemporary addiction research to study relapse-like alcohol-seeking behavior and its neural, molecular, and genetic basis (Table 1).

1.1 Models of Craving and Relapse Associated with Conditioning Factors

Alcohol-associated stimuli or events can evoke drug desire and lead to the resumption of drinking in abstinent alcoholics (Cooney et al. 1987, 1997; Eriksen and Gotestam 1984; Kaplan et al. 1985; Laberg 1986; Monti et al. 1987, 1993; Sinha 2009; Sinha et al. 2009, 2000). Studies in animals have confirmed that environmental stimuli associated with the reinforcing actions of alcohol—either by means of classical conditioning or acting as discriminative or contextual stimuli that signal drug availability—reliably elicit alcohol seeking in animals. Several animal models of alcohol seeking and conditioned reinforcement are used to elucidate the role of learning factors in relapse, to reveal the signaling mechanism that regulates specific aspects of conditioned alcohol seeking, and as a tool in preclinical medication development.

2 Conditioned Reinstatement

The initiation of drug seeking in response to alcohol-associated environmental stimuli can be demonstrated in the context of several conditioning procedures. The most prominent among these is the extinction-reinstatement model. Reinstatement refers to the recovery of an excitatory response to an extinguished stimulus produced by noncontingent exposure to the unconditioned stimulus. Conditioned reinstatement in the addiction literature refers to the resumption of responding at a previously drug-paired operandum produced by exposure to drug-associated environmental stimuli (for review, see Le and Shaham 2002; Shaham et al. 2003; Shalev et al. 2002).

An important consideration concerning the significance of learning factors in drug addiction is the role of discrete drug-paired versus discriminative or contextual stimuli. The latter category of stimuli signals the availability of a reinforcer and

Table 1 Summary of animal models that are currently most widely employed to measure relapse and craving. The table lists the individual models by the particular procedures/paradigms they employ, the corresponding experimental manipulations, the construct being measured (behavioral measure), and the actual dependent variable(s) measured as an index of alcohol seeking

Model	Manipulation	Behavioral measure	Dependent variable(s)
Conditioned reinstatement	Alcohol cues (CS) alcohol-associated contextual cues: alcohol-predictive discriminative stimuli (SD) or environmental context	Alcohol seeking	Non-reinforced responses (e.g., lever presses, nose-pokes)
Pavlovian spontaneous recovery	Alcohol-associated environmental context	Alcohol seeking	Non-reinforced responses
Operant runway	Alcohol SD	Alcohol seeking	Start time latency, time to reach goal
Conditioned place preference	Alcohol context	Alcohol seeking	Time spent in alcohol-associated environment
Priming-induced reinstatement	Noncontingent administration of alcohol, other drugs of abuse, or pharmacological agents	Alcohol seeking	Non-reinforced responses
Seeking-taking	Alcohol cues (CS)	Alcohol seeking, alcohol intake	Seeking link: non-reinforced responses Taking link: alcohol drinking
Stress-induced reinstatement	Footshock stress, pharmacological stress	Alcohol seeking	Non-reinforced responses
Alcohol deprivation effect	Alcohol deprivation (intermittent, repeated)	Exacerbation of alcohol intake	Alcohol drinking/self-administration

thereby sets the occasion to engage in behavior that brings the organism into contact with the reinforcing substance. A condition often associated with drug craving in humans is the cognitive awareness of drug availability. It has been argued, therefore, that the manner in which drug-associated contextual cues attain their incentive properties is likely to involve the predictive nature of these stimuli rather than only classically conditioned stimulus–response associations as modeled with reinstatement procedures that utilize discrete drug-paired conditioned stimuli (e.g., Ettenberg 1990, 2009; Ettenberg et al. 1996 for review). Moreover, by virtue of their presence during drug consumption, contextual cues also become associated with the rewarding effects of the drug and thus acquire incentive-motivational value (i.e., elicit memories of previous drug euphoria and the "magnitude" or value of the rewarding effect of drug consumption). Because of this dual action, these stimuli are particularly powerful in eliciting drug seeking and reinstatement.

Both response-contingent and response-noncontingent exposure to ethanol-associated contextual stimuli (or an ethanol-paired environmental context) reliably elicits recovery of extinguished responding at a previously ethanol-paired lever without further alcohol availability (Bienkowski et al. 1999a, b, 2004; Bowers et al. 2008; Burattini et al. 2006; Ciccocioppo et al. 2002, 2003a, b; Corbit and Janak 2007; Janak and Chaudhri 2010; Katner et al. 1999; Katner and Weiss 1999; Le and Shaham 2002; Radwanska et al. 2008; Zironi et al. 2006). The conditioned effects of, in particular, ethanol-predictive contextual or discriminative stimuli are remarkably resistant to extinction. It has been shown that these stimuli maintain recovery of ethanol seeking significantly above extinction levels that does not diminish when presented repeatedly under non-reinforced conditions (Cannella et al. 2009; Ciccocioppo et al. 2001, 2006) or elicit increased reinstatement with increasing abstinence duration (Bienkowski et al. 2004), a phenomenon that has been referred to as the "incubation of craving" (Grimm et al. 2001; Le and Shaham 2002). The persistence of the motivating effects of drug-associated stimuli in the animal literature resembles the long-lasting, compulsive-like nature of craving and relapse risk associated with exposure to drug cues in humans and provides experimental confirmation of the hypothesis that learned responses to drug-related stimuli are a significant factor in persistent vulnerability to relapse.

Consistent with clinical findings, reinstatement induced by alcohol cues is sensitive to reversal by opioid antagonist administration (Bienkowski et al. 1999a; Burattini et al. 2006; Ciccocioppo et al. 2002, 2003b; Katner et al. 1999). In alcoholics, naltrexone attenuates cue-induced craving (Monti et al. 1999; Rohsenow et al. 2000) and reduces relapse rates (O'Brien et al. 1996; Volpicelli et al. 1992). Moreover, excellent correspondence exists between neural mapping data in animals (Dayas et al. 2007; Zhao et al. 2006) and functional brain imaging studies in drinkers (e.g., Braus et al. 2001; George et al. 2001; Kaplan et al. 1983, 1984; Kareken et al. 2004; Myrick et al. 2004; Schneider et al. 2001; Vollstadt-Klein et al. 2011) with respect to the neurocircuitry activated by alcohol cue manipulations that, in humans, are closely linked with self-reports of craving. Conditioned reinstatement of ethanol seeking in animals, therefore, has

predictive and, possibly, construct validity as a model of craving and relapse linked to alcohol cue exposure.

2.1 Discrete Cues

The original and, until recently, most widely employed method to study the role of conditioning factors in drug-seeking behavior involves the pairing of a response producing the drug reinforcer with a brief presentation of an environmental stimulus. In this procedure, animals are trained to respond at a lever. Each response producing the drug reinforcer is contiguously paired with a brief presentation of a stimulus, such as a tone or cue light, to establish this cue as a conditioned stimulus (CS). Both the presentation of alcohol and the CS are contingent on a response. Once reliable ethanol self-administration is acquired, ethanol-reinforced instrumental responding is extinguished by withholding drug delivery and presentation of the CS. Subsequently, tests are conducted in which the degree of recovery of responding at the previously alcohol-paired lever (reinstatement), now maintained by response-contingent presentation of the CS only, is operationally defined as a measure of alcohol seeking or relapse.

2.2 Contextual Cues

This model is employed to study the effects of environmental context on the recovery of drug seeking. Here, drug availability is conditioned to stimuli (i.e., olfactory, auditory, tactile, or visual cues) present in the self-administration environment. This model has found increasing application over the past decade and is currently the most widely employed conditioned reinstatement model.

In contextual reinstatement procedures, environmental stimuli neither are paired contiguously with drug infusions, nor is their presentation contingent on a response (Contextual cue manipulations are, however, sometimes combined with the discrete cue conditioning procedures such that reinforced responses result in brief response-contingent presentation of a discrete cue [CS] in the ethanol-predictive environmental context). Owing to their predictive nature for drug availability, contextual stimuli “set the occasion” for engaging in reward seeking (i.e., to lead to the initiation of responding). Except for using context or discriminative stimuli as cue manipulations, these models are identical to the discrete cue (CS) model in terms of the training and experimental sequence, with conditioning followed by extinction in the absence of and, subsequently, reinstatement tests in the presence of the drug-associated cues. Several variants of the model exist. For example, the “basic” conditioned reinstatement model utilizes differential reinforcement of behavior in the presence of discriminative stimuli. In this procedure, during self-administration learning, responses at the operandum are

reinforced by the drug only in the presence of this stimulus. In the absence of the stimulus (or the presence of a distinctly different cue), responses remain non-reinforced. Following extinction, presentation of the ethanol-related stimulus elicits ethanol-seeking (relapse-like) behavior (e.g., Ciccocioppo et al. 2001, 2003b; Dayas et al. 2007; Katner et al. 1999; Katner and Weiss 1999; Kufahl et al. 2011; Liu and Weiss 2002b; Zhao et al. 2006). Another widely employed contextual conditioning model pioneered by Bouton and Schwartzentruber (1986) to study how the context influences extinction and resumption of learned behavior utilizes distinct environments that provide compound contextual cues (i.e., concurrent presence of olfactory, auditory, tactile, and visual cues). In this model, responding is reinforced by a given drug reinforcer in one context. Reinforced instrumental responding then is extinguished in a second context. Subjects subsequently tested in the second context show low drug seeking because the behavior was extinguished in this context. In contrast, animals tested in the first (drug-paired) context show reactivation or renewal of responding at the previously active operandum (Burattini et al. 2006; Crombag et al. 2002, 2008; Crombag and Shaham 2002; Zironi et al. 2006; for review, see Janak and Chaudhri 2010).

2.3 Neurocircuitry of Conditioned Reinstatement

Drugs of abuse have diverse pharmacological profiles and produce differential behavioral effects. Nonetheless, their conditioned effects share the common feature of activating major components of the brain incentive motivation circuit. With the use of reinstatement models, advances have been made in elucidating the neurocircuitry that mediates ethanol seeking associated with ethanol cue exposure. Consistent with findings from functional brain imaging in humans (e.g., Daglish and Nutt 2003; Goldstein and Volkow 2002; Heinz et al. 2005; Miller and Goldsmith 2001; Heinz et al. 2010; Myrick et al. 2004), animal studies that utilized c-*fos* expression as a marker of neural activation, targeted lesions, and site-specific pharmacological manipulations implicate interconnected cortical and limbic brain regions in response to drug cue-, drug priming-, and stress-induced reinstatement (e.g., Cardinal et al. 2002, 2008; Chen et al. 2011; Dayas et al. 2007; Janak and Chaudhri 2010; Kalivas and Volkow 2005; See et al. 2003; Steketee and Kalivas 2011; Topple et al. 1998; Tzschentke and Schmidt 2000; Zhao et al. 2006). Major components of this circuitry include the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), ventral tegmental area (VTA), nucleus accumbens (NAC), hippocampus, and dorsal striatum, which is thought to participate in consolidating stimulus–response habits via the engagement of corticostriatal loops. Ethanol-associated contextual stimuli elicit specific recruitment patterns within the mPFC, NAC, and hippocampus in rats, similar to those produced by other abused drugs (for discussion see Dayas et al. 2007), as well as brain activation patterns

evoked by ethanol cues in alcoholics (Grusser et al. 2004; Maas et al. 1998; Myrick et al. 2004).

In addition to activation of the corticostriatopallidal circuitry, contextual cues conditioned to ethanol produce activation of brain sites not traditionally linked to conditioned drug seeking and reinstatement. These include the medial parvocellular and magnocellular paraventricular nucleus (PVN) of the hypothalamus (Dayas et al. 2007; Zhao et al. 2006). Activation of medial parvocellular PVN neurons is positively correlated with HPA axis activation (Buller et al. 1998; Dayas et al. 1999), suggesting that alcohol cues elicit a stress-like neuroendocrine response. Activation of the magnocellular PVN by ethanol cues represents an effect that is consistent with psychological stress (Dayas et al. 1999), lending support to the hypothesis that ethanol cues produce stress-like effects (see below). In addition to influencing the HPA axis, activated PVN neurons, through descending brainstem projections, may influence autonomic responses associated with the anticipation of ethanol reward predicted by ethanol cues as observed in alcoholic subjects (Sinha et al. 2000; Stormark et al. 1995). Thus, subjective responses to alcohol cues include stress-like reactions, and these may contribute to drug seeking (in animals) and the resumption of alcohol use (in humans) elicited by these cues, given the well-established significance of stress as a risk factor for relapse (see 5.0. Models of Stress as a Risk Factor for Relapse).

3 Pavlovian Spontaneous Recovery

Pavlov (1927) was the first to describe the spontaneous recovery of responding by showing that while extinguishing a behavior across a number of days, a small significant increase in responding at the beginning of each new extinction session occurred. In addition, as part of his classic bell-salivation association experiment Pavlov described that following several extinction trials sufficient to abolish early session responding, the test subjects would again salivate in response to the ringing bell after a significant time period had elapsed after the last extinction session occurred and named this phenomenon spontaneous recovery.

Pavlovian spontaneous recovery (PSR) has been demonstrated in alcohol-preferring (P) rats (Rodd-Henricks et al. 2002a, b). Moreover, “pharmacological validation” that the anti-craving agent naltrexone decreases the expression of ethanol PSR (Rodd et al. 2004) confirmed the utility of PSR as a model of ethanol seeking and relapse. As well, the persistence of PSR in the absence of reward is thought to resemble the compulsive nature of drug abuse seen in humans (Anton 1999). PSR appears to be dependent on re-exposure to all stimuli in the environment previously associated with the reinforcer, and PSR increases with time (e.g., Rodd-Henricks et al. 2002a, b). More specifically, PSR is enhanced when a longer period of time has elapsed between the last extinction session (more than 1 week; see Rodd et al. 2004), suggesting that the forgetting of extinction learning occurs. Several lines of evidence indicate that, in fact, PSR represents a shift from

the expression of extinction learning to what was learned initially (i.e., the association between contextual cues and reward) and not an elimination of either form of learning (Bouton 1988; Brooks 2000; Brooks and Bouton 1993; for review, see Rodd et al. 2004). More specifically, it has been argued that PSR reflects a shift away from extinction learning to motivation to obtain the previously available reward, suggesting that PSR is a model suitable for studying craving-like behavior (Bouton 2002, 2004; Dhaher et al. 2010; Rodd et al. 2006).

4 The Operant Runway Model of Relapse

In this model, the time taken in a runway from a start box to a goal box where the drug is administered provides a dependent measure of drug seeking. In this procedure, a discriminative stimulus present in the start box, runway, and goal box is predictive of drug reward obtainable in the goal box, whereas a different discriminative stimulus predicts the non-availability of drug reward. Run times eventually decrease in the presence of the drug-predictive discriminative stimulus, but not the non-reward cue. Rats then are placed on extinction conditions under which the discriminative stimulus is absent and no drug is available in the goal box, with the result that runtime increases progressively. During subsequent reinstatement tests, reintroduction of the drug-paired discriminative stimulus decreases the runtime for reaching the goal box again. As well, drug availability in the goal box during extinction reduces runtime on the subsequent drug-free day. This decrease in the latency to reach the goal box as associated with these manipulations serves as a measure of relapse (Ettenberg 1990, 2009; Ettenberg et al. 1996). This model has not been utilized extensively to study specifically alcohol relapse processes but has been employed to examine the effects of ethanol on approach-avoidance conflicts in cocaine-seeking rats (e.g., Knackstedt and Ettenberg 2005; Knackstedt et al. 2006) and the effects of early ethanol exposure on ethanol seeking in adulthood (Walker and Ehlers 2009).

5 Conditioned Place Preference Models

An alternative approach to studying ethanol-seeking behavior is the conditioned place preference (CPP) model. CPP reflects the reinforcing value of ethanol by the degree to which animals seek and spend time in an environment (place preference) previously paired with the systemic administration of alcohol. Place conditioning has advantages over other procedures used to study the rewarding effects of drugs because the procedure is technologically simple and usually brief. The CPP procedure also provides an effective tool to separately examine manipulations that affect the initial learning (acquisition) of the drug-context association and manipulations that affect the performance (expression) of approach responses that

result from this learning. Moreover, CPP procedures are effective in establishing dose ranges and post-administration time profiles for ethanol's (and other drugs') reinforcing rather than aversive actions, with implications for understanding actions of the drug relevant for subsequent craving and relapse (for reviews, see Bardo and Bevins 2000; Cunningham et al. 2006; Liu et al. 2008; Schechter and Calcagnetti 1993; Tzschentke 2007).

5.1 Expression of Conditioned Place Preference as a Model of Relapse

Place conditioning procedures permit examination of the neuropharmacological substrates mediating the acquisition and expression of the conditioned reinforcing effects of ethanol (e.g., Camarini et al. 2010; Gremel and Cunningham 2007, 2008; Maurice et al. 2003; for review see Tzschentke 2007). Manipulations that interfere specifically with the *expression* of CPP, once acquired, provide information on the neural and motivating forces of the conditioned rewarding effects of ethanol leading to ethanol seeking or "craving." In contrast, interference with the acquisition of CPP is relevant for the understanding of neural mechanisms that mediate the acute reinforcing effects of ethanol, inferred by the establishment of Pavlovian associations between the ethanol reinforcer and place conditioning environment and, therefore, of lesser importance for the understanding of factors that drive the desire to obtain ethanol (craving) and relapse-like behavioral responses.

In CPP expression studies, the degree of preference for a previously ethanol-paired environment provides an index of the strength of ethanol seeking associated with the incentive-motivational effects of the previously alcohol-associated stimulus context. An issue to be considered, however, is that the expression of CPP typically is studied without an intervening period of abstinence before testing such that CPP has some limitations as a valid model of craving and relapse processes during abstinence. As well, CPP studies generally employ involuntary ethanol administration procedures. The reinforcing actions of ethanol under these conditions may differ from those associated with voluntary oral self-administration. As a result, the strength or nature of associations that are formed between ethanol and environmental stimuli may differ in CPP versus self-administration and conditioned reinstatement procedures. Moreover, the number of learning trials in reinstatement models of ethanol-seeking that involve the conditioning of the effects of self-administered ethanol with environmental stimuli typically is considerably greater than in the CPP procedure. Associations that are produced between specific environmental stimuli and ethanol are therefore likely to be weaker in the CPP model. As a result of these differences, the expression of conditioned ethanol-seeking responses may be differentially sensitive to pharmacological manipulation in CPP versus self-administration models, and it is likely that the neural substrates of contextual conditioning associated with CPP do not fully overlap with those

mediating the effects of stimuli conditioned to the reinforcing effects of actively self-administered ethanol. Finally, important species considerations apply to ethanol CPP. Typically, ethanol CPP is most effectively obtained in mice. In contrast, rats show little ethanol CPP (with the exception of genetically selected ethanol preferring lines) and often develop conditioned place aversion without prior ethanol acclimation procedures (Cunningham et al. 1993; Tzschentke 2007).

5.2 Reactivation of Conditioned Place Preference

Another contextual model of ethanol seeking is the reactivation of CPP. This procedure evolved from the traditional CPP procedure and incorporates features of the reinstatement model. Following the extinction of CPP, accomplished by pairings of vehicle rather than drug with the environment, re-establishment, technically termed *reactivation (or reinstatement) of CPP*, is produced by a drug injection. CPP reactivation procedures have been successfully applied in conjunction with abstinence manipulations following which drug injections or stress reactivate CPP (e.g., Buthada et al. 2012a, b; Itzhak and Martin 2002; Kuzmin et al. 2003; Mueller and Stewart 2000; Romieu et al. 2004; Szumlinski et al. 2002; Thanos et al. 2009; for review see Aguilar et al. 2009; Tzschentke 2007).

The CPP reactivation model incorporates all the advantages of the traditional CPP procedure, strengthened by allowing for extinction and abstinence manipulations important for the validity of the procedure as a model of craving or relapse. However, the CPP reactivation model also shares the limitations of the conventional CPP procedure discussed above. A further constraint is that the procedure does not provide a "pure" measure of conditioned reinforcement or contextual reinstatement, but rather of interactions between contextual conditioning and the effects of small "priming" doses of the drug or stress. Indeed, it has been suggested that CPP reactivation data be viewed with caution in terms of their relevance for understanding relapse processes until better information on the neurobiological mechanisms mediating this behavior is available (Aguilar et al. 2009).

6 Modeling Craving Induced by "Priming" Doses of Ethanol

It is well established that small doses of drugs of abuse, including ethanol, rather than reducing drug desire, elicit further drug craving (e.g., Jaffe et al. 1989; Ludwig et al. 1974). Moreover, in alcoholics, the first drink after abstinence is often associated with "loss of control," leading to severe intoxication and a return to continued alcohol abuse (Ludwig et al. 1974). This priming effect can readily be demonstrated in the reinstatement model following systemic administration of low alcohol doses (for review see Le and Shaham 2002). This model provides an

effective means to experimentally study the neural and molecular bases of the "loss of control" phenomenon that frequently is at the heart of the relapse process in alcoholics or people who are at risk for alcohol abuse (e.g., Ludwig et al. 1974). Moreover, the model provides a tool for investigating interactive effects of or co-dependence on different substances of abuse in the relapse process as illustrated by findings that nicotine "priming" can elicit reinstatement of ethanol seeking (e.g., Le et al. 2003).

7 Seeking-Taking Chained Schedules

Chained schedules consist of a "seeking phase" in which responses at a "drug-seeking" lever are initially required. Following completion of a response requirement or time interval in this first (i.e., seeking) link of the chained schedule, a second link is initiated by making available a "drug-taking" lever. Responses at this lever produce a drug reinforcer and presentation of a CS, followed by a time out period, whereupon the seeking link of the chain is re-initiated. The degree of conditioned drug seeking or relapse is measured by the number of seeking responses during sessions in which responses at the taking lever produce only the CS but do not result in drug availability.

7.1 Dissociation of Alcohol-Motivated Appetitive and Consumatory Behavior

In alcohol addiction research, a variant of "seeking-taking" chained reinforcement schedules is frequently used to dissociate ethanol-reinforced consummatory behavior (i.e., ethanol drinking) from appetitive behavior (i.e., responses induced and maintained by the incentive-motivational effects of ethanol-associated contextual cues (Samson et al. 1998, 1999, 2000). In this procedure, rats engage in ethanol seeking during an "appetitive phase" when they must complete a set of responses at a lever operandum without alcohol being available. The completion of a response requirement within a specific time results in the retraction of the lever and presentation of a sipper tube that contains ethanol solution, from which the rats are then allowed to freely drink for a given amount of time. This is called the "consummatory phase." Thus, responding during the appetitive phase provides a measure of the day-to-day strength of the animal's motivation to initiate and engage in ethanol-seeking behavior when exposed to the ethanol-predictive stimulus environment and can also serve as a measure of the desire to drink (Samson and Chappell 2002; Samson et al. 2003). Behavior during the consummatory phase, on the other hand, provides a measure of actual ethanol consumption as an index of the acute reinforcing strength of ethanol. In this model,

seeking and consumption are not necessarily correlated. More importantly, this model allows for the investigation of neural mechanisms that control seeking or approach responses (i.e., ethanol "craving") versus mechanisms that control the reinforcing effects of ethanol (Saghal 1984). From a drug treatment development perspective, this model provides an effective tool to evaluate the relative efficacy of a potential treatment drugs for preferential "therapeutic" actions on ethanol craving versus actual ethanol intake (e.g., Czachowski et al. 2002; Sharpe and Samson 2001).

7.2 *Chained Schedules as Potential Measures of Compulsive Alcohol Seeking*

In addition to measuring drug seeking, seeking-taking chained schedules provide a potential model of drug compulsion when combined with manipulations designed to establish the degree to which drug seeking becomes resistant to suppression by aversive stimuli. Compulsive ethanol seeking, a hallmark of substance dependence on ethanol, is characterized by its continuation despite adverse consequences (American Psychiatric Association 2000). Behavior motivated by rewards is suppressed by aversive signals, a phenomenon known as "conditioned suppression" (e.g., Bouton et al. 2008; Kearns et al. 2002; Lauener 1963). The degree to which conditioned suppression of ethanol seeking is diminished in the presence of ethanol cues on the seeking component of a chained schedule can therefore be thought of as modeling this aspect of drug compulsion. In the cocaine field, it has been confirmed that conditioned suppression decreases significantly with increasing "severity" of dependence, indicative of the development of cumpulsive drug seeking (Pelloux et al. 2007; Vanderschuren and Everitt 2004). However, this model still awaits implementation in the alcohol field.

8 Models of Stress as a Risk Factor for Relapse

Stress has an established role in alcohol abuse in humans and is a major determinant of relapse (Brown et al. 1995; Marlatt 1985; McKay et al. 1995; Sinha 2000, 2001; Sinha et al. 2003; Wallace 1989). The significance of stress in alcohol consumption and reinforcement is also well documented in the animal literature. Stressors can facilitate the acquisition or increase the self-administration of alcohol (e.g., Blanchard et al. 1987; Higley et al. 1991; Mollenauer et al. 1993; Nash and Maickel 1988) and reliably elicit reinstatement of ethanol seeking in animal models of relapse (e.g., Le et al. 1998, 1999, 2000, 2011a, b; Zhao et al. 2006; Liu et al. 2002, 2003; Martin-Fardon et al. 2000; Sidhpura et al. 2010).

Studies of stress-induced reinstatement typically are conducted using the extinction-reinstatement model with footshock stress having been the predominant model. More recently, pharmacological stressors have been employed as an alternative to footshock.

8.1 Footshock Stress

To study stress-induced ethanol seeking in the reinstatement model, rats are trained to self-administer ethanol. Once stable ethanol self-administration is established, ethanol-reinforced responding is extinguished. The reinstatement of ethanol seeking then is studied under extinction conditions after exposure to variable intermittent electric footshock administered through the grid floor of the operant chambers. Several procedural variations have been employed such as exposure to footshock in the reinstatement test environment versus a different environment (Liu and Weiss 2002a, 2003; Le et al. 2000; Martin-Fardon et al. 2000; Sidhpura et al. 2010; Zhao et al. 2006; for review see Le 2002).

This model has been instrumental in the identification of brain regions that are recruited by stress and that may play a pivotal role in stress-induced drug seeking. These brain regions include the bed nucleus of the stria terminalis (BNST) (Erb and Stewart 1999; Shaham et al. 2000; Wang et al. 2006; Zhao et al. 2006), central nucleus of the amygdala (CeA) (Shaham et al. 2003), PVN (Dayas et al. 2007; Zhao et al. 2006), and mesocorticolimbic circuitry components, including the NAC, BLA, and VTA (Wang et al. 2005, 2007; Zhao et al. 2006). Overlap exists in the pattern of neural activation produced by footshock and exposure to ethanol-related contextual stimuli. Ethanol cue exposure, however, produces a stronger activation of brain regions linked to motivation and reward, such as the mPFC and hippocampus, versus footshock stress, whereas footshock stress induces stronger neural activation within brain stress sites and particularly the PVN (Dayas et al. 2007; Zhao et al. 2006). In addition, both footshock and ethanol-related stimuli activate the CeA and BNST (Zhao et al. 2006), a finding that may reflect possible stress or anxiety-like effects of the ethanol cue. Stress and drug cue exposure may, in fact, induce a similar pattern of neural activation as suggested by findings showing that in alcoholics craving states associated with drug cue exposure are accompanied by anxiety and HPA activation (Fox et al. 2005; Sinha et al. 2003; Sinha 2009).

8.2 Pharmacological Stressors

Recently, pharmacological stress manipulations have been developed and employed to study the role of stress in relapse using the extinction-reinstatement model. Here, a challenge injection of a pharmacological stressor is administered instead of footshock before reinstatement testing.

To date, the pharmacological stressor of choice has been yohimbine, an α_2 noradrenergic receptor antagonist that is anxiogenic and induces stress responses in both humans and nonhuman primates (Albus et al. 1992; Charney et al. 1983). Early findings revealed that yohimbine elevates drug craving and elicits opioid withdrawal symptoms in methadone-maintained patients (Stine et al. 2002). In animals, yohimbine elicits stress reflected by increased plasma corticosterone, increased arterial blood pressure, increased heart rate, and potentiation of the startle response (Davis et al. 1979; Lang and Gershon 1963; Suemaru et al. 1989), confirming that yohimbine is a suitable pharmacological agent for studying stress-induced drug-seeking behavior.

Yohimbine has since been increasingly used as an alternative stressor to footshock in animal models of drug seeking (Feltenstein and See 2006; Marinelli et al. 2007) and reward seeking (Fuchs et al. 2006; Nair et al. 2006). Yohimbine has been shown to reinstate alcohol seeking (Gass and Olive 2007; Le et al. 2011a, b, 2005; Stopponi et al. 2011a; b) as well as heroin (Banna et al. 2010), cocaine (e.g., Buffalari and See 2011; Feltenstein and See 2006; Lee et al. 2004), and methamphetamine seeking (Shepard et al. 2004).

Yohimbine-induced reinstatement has been validated as a model of stress-induced ethanol seeking or relapse using pharmacological tools. Specifically, it has been shown that “anti-stress” agents including corticotropin-releasing-factor (CRF) and hypocretin-1 receptor antagonists effectively prevent the effects of yohimbine on ethanol seeking (Richards et al. 2008; Marinelli et al. 2007). Yohimbine-induced reinstatement is now used extensively for identifying novel pharmacological targets for the prevention of stress-induced ethanol seeking (Le et al. 2011a, b; Nielsen et al. 2011; Stopponi et al. 2011a, b).

8.3 *Interactive Effects Between Stress and Conditioning Factors*

Risk factors for relapse are typically studied in isolation, whereas abstinent alcoholics are frequently exposed to multiple external risk factors while at the same time experiencing varying degrees of protracted withdrawal symptoms resulting from ethanol-induced neuroadaptive dysregulation. Important for understanding the significance of drug-related learning in the relapse process, therefore, are findings that the presentation of alcohol cues significantly exacerbates the reinstatement of alcohol seeking produced by stress. Interactive effects of stress and alcohol-related cues were modeled by testing the concurrent effects of footshock stress and an ethanol-associated CS on reinstatement under three conditions: (1) during response-contingent presentation of an ethanol CS alone, (2) after exposure to very mild footshock stress alone, and (3) during response-contingent presentation of the ethanol CS following exposure to footshock stress (Liu and Weiss 2002a, 2003). Under these conditions, the ethanol CS and footshock,

when presented alone, produced only threshold effects on reinstatement of alcohol seeking. However, the ethanol CS elicited strong reinstatement in rats that had been subjected to footshock stress before the session.

The above findings document the existence of interactive effects between two factors implicated in craving and relapse: stress and alcohol-related cues. However, these studies, similar to the majority of research on the neural basis of ethanol seeking, remained confined to ethanol nondependent rats. Rats made ethanol dependent via chronic ethanol vapor inhalation or a chronic ethanol liquid diet show deficiencies in extracellular dopamine in the nucleus accumbens that are likely linked to withdrawal-associated reward deficits (Weiss et al. 2001; Schulteis et al. 1995) as well as hypersecretion of the stress-regulatory molecule CRF in the central amygdala and BNST (Merlo Pich et al. 1995; Olive et al. 2002). The dysregulation of CRF transmission is long-lasting, as are stress and anxiety-like behavioral manifestations of this dysfunction (Zorrilla et al. 2001; Zhao et al. 2007; Valdez et al. 2002). As well, recently abnormal function of metabotropic glutamate receptors with implications for stress-induced reinstatement has been identified in rats with histories of ethanol dependence (Kufahl et al. 2011; Sidhpura et al. 2010). Given that chronic alcohol intoxication leads to profound neuroadaptive dysregulation and, as a consequence, states of negative affect that represent a substantial risk factor for relapse (Koob 2003; Koob and Le Moal 2008), animal models have been employed to investigate the impact of alcohol dependence histories on cue- and stress-induced alcohol seeking. These studies revealed that in ethanol-dependent rats, the individual effects of an ethanol CS and footshock stress on reinstatement were substantially enhanced compared to nondependent rats, and that the interactive effects of the CS and footshock were, in fact, synergistically enhanced with a nearly 300% increase in ethanol seeking (Liu and Weiss 2002a).

The significance of a dependence history with respect to its role in the effects of alcohol cues and stress is illustrated further by the finding that previously ethanol-dependent rats not only show enhanced reinstatement induced by footshock stress, but also by a CS conditioned to footshock stress as well as the interactive effects of these cues (Liu and Weiss 2003).

The existence of such interactive effects between drug cues and stress has been corroborated in the context of pharmacological stress manipulations. These studies demonstrated that yohimbine greatly potentiated cocaine- and heroin-associated cue-induced reinstatement and that the BNST is a key mediator for the interaction between stress and cues for the reinstatement of cocaine seeking (Banna et al. 2010; Buffalari and See 2011; Feltenstein and See 2006).

Overall, the findings generated with the use of these animal models suggest that the probability of relapse varies as a function of the number and intensity of risk factors operative at any given time, with relapse occurring when the sum of these motivating forces reaches a critical threshold.

9 The Alcohol Deprivation Effect (ADE) as a Relapse Model

A well-described phenomenon in the alcohol literature is a marked increase in ethanol consumption that follows periods of alcohol deprivation. Early experiments revealed that rats show marked increases in voluntary ethanol consumption after periods of forced abstinence (Sinclair 1972, 1979; Sinclair and Li 1989; Sinclair and Senter 1967, 1968). This so-called "alcohol deprivation effect" (ADE) has since been confirmed in mice (Salimov and Salimova 1993), rats (Spanagel et al. 1996; Wolffgramm and Heyne 1995), and monkeys (Kornet et al. 1990, 1991). It has also been shown that the ADE occurs under both limited and unlimited alcohol access conditions and with both home cage free drinking and operant self-administration models. However, the ADE appears most robust in the two-bottle free choice procedure using genetically alcohol-preferring animals or after extensive repeated cycles of intoxication and deprivation (Heyser et al. 1997; McBride et al. 2002; Spanagel and Holter 2000; Spanagel and Kiefer 2008). Nonetheless, the ADE is well established as a robust and reliable phenomenon in animal models of alcohol drinking.

The ADE is considered a measure of the motivation to seek and consume alcohol (Eravci et al. 1997; Rankin et al. 1979; Sinclair and Senter 1967), loss of control (Wolffgramm and Heyne 1995; Spanagel and Holter 2000), or relapse (Kornet et al. 1991; McBride and Li 1998). Similarities exist between the ADE in animals and humans, such as enhanced ethanol consumption after abstinence in social drinkers (Burish et al. 1981) and the loss of control phenomenon that surrounds the first drink after abstinence in alcoholics (Ludwig and Wikler 1974; Ludwig et al. 1974; O'Donnel 1984). In view of these similarities, the ADE has appropriate face validity as a model for alcohol relapse process (Vengeliene et al. 2009). Indeed, many consider the ADE a "true" model of relapse compared to reinstatement models that do not measure resumption of actual drug taking and, therefore, perhaps more accurately model craving rather than actual relapse. Moreover, findings that pharmacological agents that suppress ethanol intake and reduce the likelihood of relapse in humans effectively attenuate the ADE in animals further support the predictive validity of this procedure as a model of relapse (Heyser et al. 1998, 2003; McBride et al. 2002; Schroeder et al. 2005; Spanagel and Kiefer 2008).

With repeated cycles of deprivation and increased deprivation periods, increased drinking associated with the ADE appears to become resistant to manipulations of ethanol concentration, taste, and environmental factors (Spanagel et al. 1996; Wolffgramm and Heyne 1995; Vengeliene et al. 2009). More specifically, in rats given long-term (8–24 months) continuous free access to different concentrations of ethanol and water, interspersed with deprivation periods of varying lengths, ethanol consumption increases significantly over baseline as a result of deprivation episodes (Spanagel et al. 1996; Wolffgramm and Heyne 1995), reaching levels of intake similar to those in rats selectively bred for alcohol preference (Li et al. 1979). The increase in ethanol intake produced by repeated

deprivation outlasts long abstinence phases (Spanagel et al. 1996) and may become irreversible (Wolffgramm and Heyne 1995). Under these conditions, the ADE is characterized not only by enhanced preference for ethanol over water but preference for higher ethanol concentrations (>10% v/v) and resistance to modification by changes in the palatability of ethanol via quinine or sucrose addition, or by manipulation of environmental and social conditions such as isolation or changing dominance hierarchies (Spanagel et al. 1996; Vengeliene et al. 2009). Moreover, ethanol deprivation under these exposure conditions revealed a behavioral withdrawal syndrome, reflected by lowered thresholds of footshock reactivity, which reached a maximum on the second day of abstinence and persisted for up to 5 days post-ethanol (Heyne et al. 1991; Holter et al. 2000). Extending these observations, access to multiple concentrations of ethanol and exposure to multiple deprivation cycles can partially overcome the genetic predisposition of NP, LAD-1, and LAD-2 rats for low alcohol consumption. These findings support the unexpected conclusion that the genetic control of low alcohol consumption in rats is not associated with inability to develop an ADE (Bell et al. 2004).

Overall, the ADE, in particular with repeated deprivation, provides an effective model to study the development of compulsive alcohol-seeking behavior and loss of control that characterize substance dependence on alcohol. Given that the ADE can be observed under many different experimental conditions, this phenomenon may have great utility for the exploration of diverse variables that contribute to the relapse process. However, long-term repeated alcohol deprivation procedures that produce the most robust exacerbation of alcohol consumption are time and labor-intensive and have not been employed extensively.

10 Conclusions

Alcohol craving and vulnerability to relapse represent formidable challenges for the successful treatment of alcohol addiction. Increasingly sophisticated animal models of ethanol seeking and relapse have become available over the past decade and have been instrumental for expanding our understanding of the neurobiological basis of susceptibility to relapse and for studying the treatment drug potential of pharmacological agents. Nonetheless, the validity of animal models of relapse, in particular of reinstatement models, has not gone unchallenged. The literature contains both critical (e.g., Epstein et al. 2006a; Katz and Higgins 2003) and supportive (Epstein et al. 2006a, b) appraisals of these models. Taking into account both the limitations and advantages of these models, it is perhaps safe to conclude that reinstatement models are the most effective procedures available to date for investigating the neural bases of craving and relapse and for evaluating the potential of drug treatments for craving and relapse prevention. An effective model also is the "seeking-taking" chained reinforcement schedule that permits concurrent investigation of both the strength of ethanol-seeking behaviors,

presumably reflecting craving, and changes in the primary reinforcing effects of ethanol. Expression and reinstatement of conditioned place preference provide relapse models that are easy to implement and considerably less labor-intensive than conditioned reinstatement chained schedules or alcohol deprivation procedures. However, as outlined above, CPP procedures have several limitations that require consideration when evaluating data generated by these models for their relevance for understanding the relapse process. The ADE has substantial potential as a model to study the development of compulsive alcohol-seeking behavior and loss of control. However, difficulties in reliably obtaining an ADE with operant ethanol self-administration procedures and the longitudinal nature of repeated ADE procedures required to obtain the most robust effects somewhat limit the utility of this model.

Clearly, many animal models are available that permit investigation of alcohol-seeking behaviors (craving), the resumption of ethanol consumption following abstinence (relapse, loss of control), as well as neurobehavioral mechanisms and genetic factors that regulate these behaviors. These models also provide valuable tools for identifying pharmacological treatment targets and for evaluating the efficacy of potential treatment drugs for alcohol craving and relapse. At the same time, several important issues for improvement and advancement in our animal model repertoire exist. It will be important to establish the constructs measured by particular models in order to more effectively employ these procedures to study specific aspects or stages of the alcohol addiction cycle. In particular with regard to medications development, a need exists to establish the predictive validity of existing models. Pharmacological agents often do not produce the same modifications in ethanol-seeking behavior across these models, and it will be important to understand the implications of these differences for understanding both the construct measured by a given model and its predictive validity.

Perhaps the biggest challenge for the development and refinement of animal models for craving or relapse is the issue of ethanol dependence history. With the exception of animals genetically selected for high ethanol intake, most animals will not voluntarily consume ethanol at levels sufficient to induce dependence. Repeated and long-term alcohol deprivation procedures can accomplish this, but the longitudinal nature of these procedures renders them impractical for everyday applications. Some progress with achieving high voluntary ethanol intake has been made with intermittent ethanol access procedures (Simms et al. 2008) that lead to high and quinine-resistant ethanol intake (Hopf et al. 2010) and may provide an avenue to incorporate dependence-like drinking into existing models of relapse, in particular the reinstatement and chained schedule models. The dependence history issue is of special relevance for the understanding of craving and relapse associated with conditioning factors. Present behavioral and neurobiological information on the role conditioning factors in ethanol seeking is limited largely to that from animal studies in nondependent subjects. In alcoholics, a significant positive correlation exists between history of dependence and the severity of cue-induced ethanol craving (Greeley et al. 1993; Laberg 1986; Myrick et al. 2004; Streeter et al. 2002). Thus, cue-induced ethanol seeking in animals without histories of

dependence is unlikely to effectively model the learning events and motivating forces that underlie the compulsive nature of ethanol seeking in alcoholics with long histories of heavy drinking and repeated episodes of withdrawal. Ethanol consumption during withdrawal modifies an individual's reinforcement history to include learning about amelioration or avoidance of adverse withdrawal states as a novel and essential aspect of alcohol's reinforcing actions, rendering the drug a qualitatively different, more potent reinforcer. Thus, understanding the control of behavior by stimuli conditioned to ethanol under conditions that encompass the reinforcing dimension of this drug that emerges with the experience of withdrawal states will be essential for advancing the understanding and treatment of alcohol addiction.

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Invertebrate Models of Alcoholism

Henrike Scholz and Julie A. Mustard

Abstract For invertebrates to become useful models for understanding the genetic and physiological mechanisms of alcoholism related behaviors and the predisposition towards alcoholism, several general requirements must be fulfilled. The animal should encounter ethanol in its natural habitat, so that the central nervous system of the organism will have evolved mechanisms for responding to ethanol exposure. How the brain adapts to ethanol exposure depends on its access to ethanol, which can be regulated metabolically and/or by physical barriers. Therefore, a model organism should have metabolic enzymes for ethanol degradation similar to those found in humans. The neurons and supporting glial cells of the model organism that regulate behaviors affected by ethanol should share the molecular and physiological pathways found in humans, so that results can be compared. Finally, the use of invertebrate models should offer advantages over traditional model systems and should offer new insights into alcoholism-related behaviors. In this review we will summarize behavioral similarities and identified genes and mechanisms underlying ethanol-induced behaviors in invertebrates. This review mainly focuses on the use of the nematode *Caenorhabditis elegans*, the honey bee *Apis mellifera* and the fruit fly *Drosophila melanogaster* as model systems. We will discuss insights gained from those studies in conjunction with their vertebrate model counterparts and the implications for future research into alcoholism and alcohol-induced behaviors.

H. Scholz (✉)
Department for Animal Physiology, University of Cologne Biocenter,
Zülpicher Straße 47b, 50674 Köln, Germany
e-mail: hscholz2@uni-koeln.de

J. A. Mustard
School of Life Sciences, Arizona State University, PO Box 874501,
Tempe AZ 85287, USA

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Abbreviations

ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
GABA	Gamma-aminobutyric acid
NPY	Neuropeptide Y
NPF	Neuropeptide F
PKA	cAMP Dependent protein kinase A
EGF	Epidermal growth factor
GRASP	Green-fluorescent protein function across synaptic partners
QTL	Quantitative trait loci
DSM-IV	Fourth edition of the diagnostic and statistical manual of mental disorders

Contents

1 Ethanol Metabolism in Flies, Worms, and Honey Bees 434
2 Ethanol Induced Behaviors in Invertebrates 436
3 The Molecular Basis of Intoxication in Invertebrates 439
3.1 Neurotransmitter Systems 439
3.2 Neuropeptides 440
3.3 Synaptic Machinery 441
3.4 Channels 442
3.5 Second Messenger Pathways 442
4 Advantages of Using Different Invertebrate Models 443
5 Comparison of Criteria Used to Classify Alcoholism and Behavioral Paradigms Used in Invertebrates 447
5.1 Tolerance 447
5.2 Withdrawal 449
5.3 Chronic Increases in the Consumption of Alcohol Over Time, Inability to Control Alcohol Use, and Excessive Amounts of Time Spent with Alcohol ... 449
5.4 Other Behaviors Associated with Alcoholism 450
6 Conclusions 451
References 452

1 Ethanol Metabolism in Flies, Worms, and Honey Bees

Ethanol is produced in very small amounts in almost all living organisms as a metabolic by-product (Holmes 1994). In addition, in the natural environment ethanol concentrations of up to 5% can be found in fleshy fruits (Dudley 2002;

Gibson and Oakeshott 1981). Furthermore ethanol vapor is emitted from woods like eucalypts (Maleknia et al. 2009). Ethanol-enriched food sources are preferred by some invertebrates. For example, the ambrosia beetle is attracted to ethanol-containing volatiles from trees and nymphalid butterflies prefer fermenting fruits (Hill et al. 2001; Ranger et al. 2010). In addition, a number of plant species use fermented nectar to attract both mammalian and invertebrate pollinators (Wiens et al. 2008; Goodrich et al. 2006). In this context ethanol as an odor might serve primarily as a long range signal for localization of a transient food source (Dierks and Fischer 2008). The three invertebrate models for alcoholism-related behaviors: the nematode *Caenorhabditis elegans* (*C. elegans*), the fruit fly *Drosophila melanogaster* (*Drosophila*) and the honey bee *Apis mellifera* discussed in this chapter are exposed to low internal ethanol concentrations, but also to higher concentrations of ethanol in their environment. *C. elegans* live and feed in soil filled with nutrients and microbiologically active organic material. They dwell in man-made environments such as compost and garden soil that are also fermenting and producing increasing concentrations of ethanol. Adult *Drosophila* flies are attracted by the smell of ethanol-containing food sources (Ogueta et al. 2010) and female flies prefer to lay their eggs on ethanol-containing media (McKenzie and Parsons 1972). As well as encountering ethanol in fermented nectar, the honey bee may encounter ethanol inside the hive as numerous yeasts, including *Saccharomyces cerevisiae* have been isolated from pollen stores suggesting that ethanol might be produced in low levels (Gilliam 1979). Furthermore, under humid conditions honey stored in the hive may ferment exposing the bees to even higher ethanol concentrations.

Ethanol is normally toxic and is commonly degraded by Alcohol dehydrogenase (ADH), an enzyme that is present in almost all living animals (Holmes 1994). ADH is required for the synthesis and the degradation of ethanol. In yeast, under anaerobic conditions, ADH catalyzes the final step of fermentation that leads to the production of ethanol and NAD^+. In the presence of ethanol, ADH converts ethanol to acetaldehyde. Acetaldehyde in turn is transformed by the enzyme Aldehyde dehydrogenase (ALDH) to acetyl-CoA. However, at least one alternative ethanol-degrading pathway exists. In *Drosophila* larvae 90% of the ethanol degrades via ADH, but the residual ethanol is metabolized by alternative enzymes such as Catalases (Geer et al. 1985, 1993). The ADH gene has been studied extensively in *Drosophila*. Adult flies with impaired ADH function accumulate more ethanol than control flies (Wolf et al. 2002) suggesting that ADH is one of the major enzymes of ethanol metabolism. The increase in ethanol levels is accompanied by changes of behavior. ADH mutants show a reduction in the locomotor activating effect of ethanol, and an increased sensitivity and negative tolerance to the effect of ethanol on postural control (Wolf et al. 2002; Ogueta et al. 2010). Impaired ADH function is also correlated with a loss of preference to low concentrations of ethanol and reduced aversion to high ones (Ogueta et al. 2010). The conversion of acetaldehyde to acetyl-CoA is also important, since flies with impaired ALDH function show a reduction in survival when exposed to ethanol compared to controls (Fry and Saweikis 2006).

In the free living nematode *C. elegans*, the activity of ADH has been demonstrated (Bolla et al. 1987). ADH mutants have been isolated based on the observation that mutants without ADH activity are resistant to allyl alcohol (Williamson et al. 1991). In addition, at least two members of the Zn-containing alcohol dehydrogenase family of enzymes have been isolated (Glasner et al. 1995). Furthermore, the genome of *C. elegans* contains three *Catalase* (CTL) genes in tandem: CTL-3, CTL-1, and CTL-2 (Petriv and Rachubinski 2004).

ADH activity has also been detected in the honey bee (Martins et al. 1977, Bouga et al. 2005). Thus, in principle, flies, bees, and worms are all well equipped to degrade ethanol utilizing pathways similar to those found in other mammalian models such as rats and mice. Therefore, these invertebrate models also provide the opportunity to investigate the consequences of an altered ethanol metabolism on ethanol-induced behaviors. However, future work needs to be done to better characterize the major routes of ethanol metabolism in *C. elegans* and honey bees.

2 Ethanol Induced Behaviors in Invertebrates

In vertebrates, including humans, an acute internal dose of ethanol at low concentration leads to hyperactivity, while higher concentrations cause uncoordinated motor function and immobility. To be a useful model for studying the mechanisms underlying the action of ethanol invertebrates should display similar signs of intoxication as mammals, preferably at similar ethanol doses.

Already in the late 1960s, it was observed that adult flies with impaired ADH activity could not fly or walk properly, and eventually became sedated when exposed to media containing ethanol (Grell et al. 1968). When first exposed to ethanol vapor, wild-type flies show an increase in locomotion due to an odor-evoked startle response. Under continuous exposure this response is followed by a second phase of hyperactivity accompanied by measurable increases in internal ethanol concentrations. Eventually, flies reduce their locomotor activity and become sedated (Singh and Heberlein 2000; Wolf et al. 2002). Aspects of these behaviors can be characterized in detail using an array of assays. The different phases of activity and the degree of sedation can be measured with a locomotor tracking system and a loss of righting test (Singh and Heberlein 2000, Park et al. 2000; Wolf et al. 2002; Godenschwege et al. 2004; Rothenfluh et al. 2006; Ramazani et al. 2007). The hyperactive phase can be analyzed using a simple line crossing assay where the number of times a fly crosses a line or breaks a light beam reflects its speed (Bainton et al. 2000; Singh and Heberlein 2000; Parr et al. 2001). The degree of sedation can be measured using the *inebriator*, a test system consisting of tubes that are perfused with ethanol vapor until the flies stop moving. The time required for recovery reflects the level of sedation (Cowmeadow et al. 2005). In addition to changes in locomotion flies lose their postural control during ethanol exposure. In part this behavior might be due to uncoordinated hyperactivity or increased sedation (Moore et al. 1998; Wolf et al. 2002; Rodan et al. 2002).

The effect of ethanol on postural control can be measured with a device called the *inebriometer* (Cohan and Hoffman 1986; Moore et al. 1998). The *inebriometer* consists of a 1.22 m long column filled with ethanol vapor. A population of flies is inserted into the top of the column where they are exposed to the ethanol vapor, which they breathe resulting in increasing levels of ethanol. With prolonged external exposure flies start to lose their postural control and tumble down the column. At the bottom of the column they are counted as they pass through a light beam. The average time spent in the column is used as an indicator for the sensitivity of the flies to the effect of ethanol on postural control. Upon removal of ethanol delivery, the flies recover from sedation (Berger et al. 2004; Wen et al. 2005).

After a recovery phase followed by a second ethanol exposure, flies develop tolerance to the odor-evoked startle response, display an increase in ethanol-induced hyperactivity and show tolerance to the locomotor-repressing effect of ethanol (Scholz et al. 2000; Scholz 2005). In addition they develop tolerance to the effect of ethanol on postural control (Scholz et al. 2000). Rapid and chronic tolerance can also be observed for ethanol-induced sedation, as after one or more initial exposures, the same dose of ethanol leads to fewer flies being sedated (Godenschwege et al. 2004; Urizar et al. 2007). In addition, flies receiving repetitive or chronic doses of ethanol recover more quickly from sedation (Berger et al. 2004; Cowmeadow et al. 2005). The internal ethanol concentration accompanying the observed behavioral changes is ~15 mM for the onset of the locomotor repressing effect of ethanol and ~30 mM for the loss of postural control (Wolf et al. 2002; Scholz et al. 2000). After recovering from an initial dose of ethanol for 4 h, flies need around 40% more ethanol (~42 mM) before losing postural control. These concentrations are comparable to concentrations that cause intoxication in non-addicted humans (Scholz et al. 2000).

In response to ethanol, wild-type *C. elegans* show a repertoire of behaviors similar to those observed in *Drosophila*. To treat *C. elegans* with ethanol, doses of ethanol are added to the media on which they live leading to increasing internal ethanol concentrations, the longer they are exposed to the media. Initially, the nematodes increase their movements on an agar plate containing ethanol. This phase is followed by a loss of coordination and eventually immobility as internal ethanol concentrations continue to rise. Finally they no longer respond to a tap on the snout - a stimulus normally resulting in backing behavior. This state is referred to as anesthesia. The immobility is reversible on removal of ethanol. The ethanol treatment does not influence life span, fertility, and movement, feeding or mating (Morgan and Sedensky 1995). Low external doses of ethanol ranging from 0.1 to 0.3% or 17.4 to 52.5 mM cause hyperactivity, whereas higher concentrations of 0.5–5.15% or 87–870 mM decrease motility (Eckenhoff and Yang 1994; Morgan and Sedensky 1995; Dhawan et al 1999; Graham et al. 2009). A detailed analysis of the amplitude of body bends during locomotion showed that concentrations from 100 to 500 mM ethanol in the media reduces the rate of body bends, decreases the speed of locomotion and depresses the frequency of egg-laying in a dose dependent manner. At these concentrations, hyperactivity was not observed

(Davies et al. 2003). At high ethanol concentrations nematodes are immobile within 10 min. However, even after a 6 h exposure, the immobility is still reversible. It has been suggested that this complete paralysis reflects the anesthetic action of ethanol (Hong et al. 2008). Acute ethanol tolerance is also observed in *C. elegans*. After 10 min of exposure to 500 mM ethanol, nematodes show a depression in locomotor activity. However, over time the speed of locomotion increases again even though internal ethanol concentrations remain constant suggesting that nematodes do indeed develop acute tolerance (Davies et al. 2004).

The internal ethanol concentrations causing these behavioral changes are thought to be roughly 1/10 of the external ethanol concentration, possibly due to the fact that the hypodermis of the nematodes functions as a barrier (Davies et al. 2003). If this is the case, then the internal ethanol concentrations reached are comparable to concentrations that cause intoxication in humans (Davies et al. 2003). However, this observation is under debate since other results indicated higher internal concentrations corresponding more closely to the external ethanol concentration of the media (Mitchell et al. 2007). The differences in measured internal concentrations may reflect the different protocols used to determine internal ethanol concentrations. Despite the debate over internal ethanol concentrations, *C. elegans* clearly shows intoxication in a dose dependent manner similar to vertebrates. The use of *C. elegans* to identify new molecules involved in the regulation of ethanol-induced locomotor depression (described below) proves that this organism is a successful model for examining the molecular mechanisms underlying the actions of ethanol. However, the relationship between external and internal ethanol concentrations needs to be further investigated.

Honey bees willingly consume ethanol. With the sucrose concentration held at 1 M (approximately 33%), honey bees will ingest solutions containing up to 50% ethanol (Maze et al. 2006). Initial studies using crossing in a shuttle box or the turning of a running wheel, suggested that ethanol consumption by bees reduced walking behavior (Abramson et al. 2000). A more detailed analysis using the observation of individual bees in an arena revealed time and dose-dependent changes in motor function, e.g., a decrease in the walking time, a loss of postural control and loss of the righting reflex. However, a hyperactive locomotor phase was not observed, possibly because bees in the arena spent the majority of the time walking, even in the absence of ethanol (Maze et al. 2006). Given the larger size of the honey bee, blood ethanol levels could be measured directly from hemolymph samples (Maze et al. 2006; Bozic et al. 2007). Hemolymph ethanol levels associated with these changes in behavior were 25–100 mM, similar to those observed in other animals exhibiting similar behaviors (Maze et al. 2006). After consumption of ethanol, hemolymph ethanol levels increase for 30–60 min in a time and dose-dependent manner (Bozic et al. 2007; Maze et al. 2006). The levels remain fairly constant for several hours before decreases are observed. One possible mechanism for the delayed ethanol metabolism of ethanol might be that solutions ingested by honey bees are stored in the crop, the storage structure which foraging bees use to carry nectar and water back to the colony. Passage of material from the crop into the gut is regulated by hemolymph sugar levels; when sugar

levels drop, the proventriculus opens allowing solution to flow from the crop into the gut (Blatt and Roces 2002). It is possible that the slow release of the ethanol containing sucrose solution from the crop into the gut is responsible for the relatively long time course observed for ethanol metabolism. Studies using an ethanol–water vapor delivery system similar to that used in *Drosophila* showed that bees changed their locomotor behavior in a similar manner as bees that ingested ethanol. The changes in behavior included decreases in walking, increases in grooming behavior, uncoordinated movements, and extension of the proboscis. In contrast to bees fed with ethanol, the recovery of normal locomotor behavior was quite rapid (approximately 10 min) once the ethanol vapor was removed (Ammons and Hunt 2008a, 2008b). This is consistent with the model that the prolonged time needed to recover from ingestion of ethanol is due to retention of solutions in the crop, rather than honey bees having a slower ethanol metabolism. However, more work needs to be done to characterize ADH activity and the movement of ethanol from the crop into the gut. As with other invertebrates like nematodes and flies, ethanol affects locomotion in honey bees in ways that are analogous to its effect in mammals.

3 The Molecular Basis of Intoxication in Invertebrates

Beside behavioral similarities between invertebrate and mammalian model systems, invertebrates also use similar neurotransmitter systems, neuropeptide, synaptic proteins, channels and signaling processes to mediate ethanol-induced behaviors. Examples for these molecules and signaling processes are described in the following sections.

3.1 Neurotransmitter Systems

Dopamine signaling has been implicated in alcohol abuse and the development of alcoholism. In particular, dopamine is involved in mediating the rewarding properties of a drug (Heinz 2002, among others). On a behavioral level addiction has been associated with the acute locomotor stimulating effect of ethanol (Wise and Bozarth 1987). Pharmacological manipulations reducing dopamine concentrations and signaling in flies also cause a reduction of ethanol induced locomotor activity (Bainton et al. 2000). Recent analyses identified specific dopaminergic neurons in the brain that mediate the enhanced locomotor activity. Interestingly, these neurons project to the central complex—a structure implicated in the regulation of motor behaviors in insects (Kong et al. 2010; Strauss 2002). Knock out of a D1-like dopamine receptor, DmDOP1, present in the central complex leads to a loss of ethanol-induced locomotion consistent with the finding that dopamine

signaling is required for the activation of ethanol-induced locomotor activity (Kong et al. 2010).

Ethanol preference is a measurement of how much an animal favors ethanol. When placed on agar plates containing ethanol, *C. elegans* develops preference to ethanol within 4 h. Naïve worms avoid 300 mM ethanol containing media, whereas pre-exposed worms will move to regions of the agar plate containing ethanol. The degree of preferences depends on the pre-expose time and internal ethanol concentrations. Animals raised in ethanol-containing media show the highest preference. Ethanol preference develops also when during the first exposure no food is present, however, not to the same extent. Analyses with mutants with reduced levels of dopamine suggest that the development of preference requires dopamine signaling (Lee et al. 2009).

Preclinical and clinical studies have implicated the gamma-aminobutyric acid (GABA) B receptor in alcohol dependence. In rats the GABA B receptor regulates alcohol intake and the motivational properties of ethanol. In alcoholics GABA B receptor function is involved in reducing alcohol withdrawal syndromes and craving (Colombo et al. 2004). In *Drosophila* it has been shown that antagonists of the GABA B receptor reduce the motion-impairing effect of ethanol (Dzitoyeva et al. 2003) suggesting that GABA signaling also plays an important role in the regulation of direct ethanol action on the central nervous system in invertebrates.

There might be also possible limitations when comparing neurotransmitter systems involved in ethanol-induced behaviors across taxa. The biogenic amines octopamine and tyramine are major neurotransmitters in invertebrates, whereas they are only found at low levels in mammals so that they are referred to as “trace amines.” Octopamine is involved in the regulation of ethanol tolerance in *Drosophila* (Scholz et al. 2000; Scholz 2005). So is there is a functional correlate in vertebrates? Some evidence suggests that many of the functional roles of noradrenalin in vertebrates are carried out by octopamine in insects (Davenport and Evans 1984). This is consistent with the finding that mice without noradrenalin do not develop tolerance (Tabakoff and Ritzmann 1977). However, there is a growing awareness that “trace amines” such as octopamine and tyramine may play important roles in mammalian brain too. For example, specific receptors for tyramine and octopamine have been characterized in mammals (Borowsky et al. 2001; Premont et al. 2001) and octopamine and tyramine may play a role in number of neurological disorders (Berry 2004).

3.2 Neuropeptides

In vertebrates, neuropeptide Y (NPY) signaling plays a role in alcohol intake and dependence (Thorsell 2007). Invertebrates have an ortholog to NPY, neuropeptide F (NPF), and signaling via NPF also influences ethanol-related behaviors. For example, flies with altered NPF signaling are more resistant to the locomotor repressing effects of ethanol (Wen et al. 2005). Furthermore, in *C. elegans,* an

NPY like receptor is implicated in the development of acute tolerance (Davies et al. 2004). Naturally occurring strains of *C. elegans* show a variation in acute tolerance caused by a mutation in a Neuropeptide Y receptor-1 (NPR-1) like protein. The NPR-1 receptor is also involved in the regulation of the velocity of locomotion and aggregation with other animals on food, a form of social behavior in *C. elegans* (de Bono and Bargman 1998). The function of NPR-1 in the regulation of food related behaviors can be genetically separated from its function in acute tolerance, since different NPR-1 expressing neurons mediate these behaviors (Davies et al. 2004). Therefore, it would be interesting to investigate whether ethanol intake can be separated from behaviors like acute tolerance in vertebrates as well.

3.3 Synaptic Machinery

In invertebrates, components of the synaptic machinery play important roles in ethanol-induced behaviors. In *C. elegansrab3* mutants are more active at high concentrations of ethanol that normally cause reduced locomotion in wild-type animals. The *rab3* gene encodes a small G protein that directly interacts with synaptic vesicles when bound to GTP. The hydrolysis of GTP leads to the transition from docking to fusion of the synaptic vesicles and promotes their release (Fukuda 2008). Consistent with the observed phenotype of *rab3* mutants, loss of function mutants of the GTP-exchange factor show reduced mobility when exposed to ethanol. Interestingly, mice without *rab3* function recover after intoxication more quickly from an ataxic ethanol dose as judged by their ability to balance on a stationary dowel. In addition, these mice increase their consumption of solutions containing from 14–20% ethanol (Kapfhamer et al. 2008). Another *C. elegans* strain with increased resistance to the locomotor repression effect of 300 mM ethanol has a specific *unc-18*^{D214N} mutation. Interestingly, at lower concentrations these mutants show a complete loss of ethanol-induced hyperactivity. The gene mutated in these nematodes—which encodes the (nSEC1/Munc18-1) protein *Unc-18*— is an important part of the pre-synaptic SNARE complex, a complex that is involved in neurotransmitter release from synaptic vesicles. The phenotypic analysis of *unc-18* mutants shows that pre-synaptic events are also sensitive to the action of ethanol (Graham et al. 2009).

The Homer protein has also been implicated in the regulation and maintenance of synaptic structures and/or plasticity and neuronal development in both mammals and invertebrates (Foa and Gasperini 2009). In *Drosophila, homer* mutants are more sensitive to the effects of ethanol and are impaired in their ability to develop rapid tolerance to the sedating effect of ethanol (Urizar et al. 2007). Furthermore, a screen in *C. elegans* focusing on mutants that are more resistant to ethanol-induced immobility lead to the isolation of nine *judang* (*jud*) mutants. The name for these mutants is derived from the Korean word *judang* that means *being tolerant to alcohol.* One of these mutants -*jud-44*- carries a mutation in a protein that shares homology to the mammalian Homer protein (Hong et al. 2008).

Therefore, it is not surprising that other molecules implicated in synaptic plasticity like Synapsin are also involved in the regulation of ethanol tolerance in *Drosophila* (Godenschwege et al. 2004).

3.4 Channels

A molecule implicated in the direct action of ethanol in *C. elegans* and *Drosophila* is the large conductance BK-type Ca^{2+}-activated K^{+} channel, which is also present in vertebrates (Brodie et al. 2007). Altered BK channel function causes hyperactive neurotransmission, which has consequences for ethanol-induced behaviors as revealed by phenotypic analyses of BK channel mutants in *C. elegans* and *Drosophila* (Davies et al. 2003; Cowmeadow et al. 2005, 2006; Ghezzi et al. 2010). In *C. elegans* and *Drosophila,* the *slowpoke-1* (*slo-1*) gene encodes the BK channel. In *C. elegans slo-1* loss of function mutants show a delay in the locomotor repressing effect of ethanol (Davies et al. 2003). On the other hand, gain of function mutants display behaviors associated with intoxication even in the absence of ethanol (Davies et al. 2003). In *Drosophila*, *slo1* mutants do not develop rapid ethanol tolerance to the sedating effect of ethanol (Cowmeadow et al. 2005). After sedation with ethanol, flies show an increase in *slo1* expression suggesting that the BK channels are required for the development of tolerance. Furthermore, a brief pulse of *slo1* expression leads to a quicker recovery from sedation suggesting that Slowpoke induction mimics a pre-exposure of ethanol in flies (Cowmeadow et al. 2006). These results suggest that it would be of interest to further analyze the relationship of BK function in the locomotor repressing effects of ethanol and/or tolerance to the recovery from the sedation effect. The BK channels are also involved in mediating ethanol-induced behavior in vertebrates, e.g., acute tolerance (Treistman and Martin 2009).

3.5 Second Messenger Pathways

In addition to neurotransmitter systems, signaling processes that play important roles in mediating ethanol-induced behaviors are also highly conserved. In vertebrates ethanol activates cAMP signaling (Diamond and Gordon 1997). Similarly, in flies a decrease in cAMP levels associated with mutations in the adenylyl cyclase Rutabaga causes an increase in sensitivity to the effects of ethanol on postural control and to the locomotor-repressing effect (Moore et al.1998; Wolf et al. 2002). Comparable phenotypes of increased sensitivity to ethanol-induced sedation can be observed in mice with impaired adenylyl cyclase 1 and 8 functions (Maas et al. 2005). In *Drosophila*, inhibition of the cAMP dependent protein kinase A (PKA) function by expression of a mutated type I regulatory subunit (PKA-RI) in a specific subset of neurons in the brain, leads to increases in resistance to the effect of ethanol on postural control and its repression of locomotor activity (Rodan et al. 2002). Furthermore, flies with a non-functional type II

cAMP-dependent protein kinase regulatory subunit (PKA-RII) are more resistant to the sedating effect of ethanol (Park et al. 2000). Interestingly, inhibition of PKA-RI signaling in insulin-producing cells has the opposite effect causing an increase in ethanol sensitivity (Corl et al. 2005). These findings suggest that cAMP signaling in different sets of cells might cause different ethanol-induced behaviors.

In addition to cAMP signaling, other signaling cascades including the epidermal growth factor (EGF) signaling cascade are involved in the regulation of ethanol-induced sedation. *Drosophila happy hour* mutants show an increase in resistance to the sedating effect of ethanol. Happy hour is a member of the Ste20 family of kinases that negatively regulate EGF/ERK signaling. Remarkably, inhibition of EGF receptor with erlotinib alters ethanol-induced behaviors in flies, mice, and rats. Flies show an increased sensitivity to the loss of righting effect, mice a reduction in recovery from sedation, and rats a reduced ethanol intake in a two-bottle choice drinking paradigm (Corl et al. 2009). In summary, there appear to be a number of conserved signaling processes in invertebrates and vertebrates that mediate ethanol-induced behaviors. Studies using microarrays to examine the differences in gene expression in alcohol preferring versus alcohol non-preferring rats implicated many of the processes described above such as cAMP signaling, signal transduction and synaptic machinery (Sommer et al. 2006). These results lead the authors to suggest that selection for ethanol preference in rats over many generations appears to work on two major biological processes, signal transduction and metabolism. This suggestion is in agreement with the forward and reverse genetic studies in invertebrates discussed above and confirms that the biological processes underlying the effects of ethanol on mammals and invertebrates are highly conserved.

4 Advantages of Using Different Invertebrate Models

In order to understand drug-induced behaviors and the transition from normal to abusive drinking, it is important to identify the molecular targets of ethanol on neurons and the neuronal mechanisms underlying different behaviors associated with alcoholism. Model organisms like *Drosophila, C. elegans* and the honey bee offer advantages and new approaches for these kinds of analyses. This section will highlight some examples where the use of these invertebrates may provide important insights into the actions of ethanol. Common advantages of all of these models include the large number of individual animals that can be used in experiments, their short generation time, and the relative simplicity of their nervous systems in comparison to vertebrates. For example, the human brain is comprised of 85 billion neurons, while the honey bee brain contains 850,000 neurons, the *Drosophila* brain 100,000 neurons, and the entire nervous system of *C. elegans* consists of 302 neurons.

The major advantage of using *Drosophila* is the whole battery of genetic tools available for manipulating brain function and behavior. Unbiased screens can be

used to knock out nearly all genes, and the behavioral consequences can be analyzed. In this context, the insertion of transgenes to produce mutant lines has facilitated the identification of the genes affected. In general, a broad set of transgenes can be used to both knock out and transfer genes back into the organism. For example, the use of the UAS/GAL4 system in *Drosophila* allows for the expression of transgenes under spatial and temporal control (Jones 2009) just as in mice carrying the Cre/loxP system. Identifying which neurons mediate a behavior and how neurons form networks is important for our understanding of behavior generation. For this purpose in *Drosophila* specific neurons can be ablated, silenced, or activated. Using these techniques, neurons and brain structures underlying ethanol-induced behaviors in *Drosophila* have been identified (Scholz 2009). Neuronal activity in response to stimuli can be monitored and the morphology of neurons can be analyzed in detail (Jones 2009; Luo et al. 2008, Olsen and Wilson 2008, among others). The analysis of the connectivity of neurons has been further facilitated by the introduction of a second transgene system similar to the UAS/GAL4 system, the binary LexA/LexAop system (Lai and Lee 2006). Furthermore, a technique based on reconstitution of Green-fluorescent protein function across synaptic partners (GRASP) allows detection of cellular contact and synapse formation in vivo and in vitro (Feinberg et al. 2008; Gordon and Scott 2009).

One big advantage of using *C. elegans* as a model is that each of the 302 neurons and approximately 5,000 chemical synapses in *C. elegans* has been characterized and its connectivity mapped by electron microscopy analysis (Sulston and Horvitz 1977; White et al. 1986). In addition, the developmental fate of every cell is known (Sulston et al. 1983), making *C. elegans* an excellent model system for examining the effects of ethanol on development (Davis et al. 2008). Elegant tools also exist for manipulating the development and genetics of nematodes. For example, specific cells can be ablated during development using a laser, and since the developmental linage is known, ablation at different times effects different cell groups. In addition, RNA interference (RNAi) can be used to knock down gene expression. Furthermore, despite the fact that nematodes have a relatively simple nervous system, they can perform an astonishing array of behaviors including both associative and non-associative learning (Giles and Rankin 2009; Saeki et al. 2001). Another advantage of *C. elegans* is their small size and short reproductive cycle of around 68 h (Wood 1988) that allow large mutagenesis screens to be carried out in relatively short times.

Several screens using different paradigms have already been performed that led to the isolation of new mutants with defects in ethanol sensitivity. In one screen for ethanol-resistant mutants, nematodes are exposed to 400 mM ethanol on an agar plate surrounded by a bacteria lawn, as a food source. After incubation for 30 min, all nematodes that are still able to crawl towards the food source are scored as resistant. A second screen does not use bacteria as a source of attraction, but utilizes an attractive odor instead (Davies and McIntire 2004). Similar screens using ethanol-induced immobility as an endpoint, led to the discovery of several genes that play a role in ethanol-induced anesthesia (Morgan and Sedensky 1995).

Mutations in the *unc79* gene cause an increase in resistance to anesthesia, whereas mutation in *fc20, fc21, fc34, fc23,* or *fc30* show an increase in sensitivity. The *fc21* mutation is in the general anesthetic sensitive gene (gas) that codes for a subunit of the nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase (complex I) of the mitochondrial electron transport chain (Kayser et al. 2001).

Although lacking the extensive genetic tool kit that can be employed in *Drosophila*, molecular and pharmacological tools are available in the honey bee for analyzing the mechanisms underlying behavior. For example, as with flies and *C. elegans*, the honey bee genome has been sequenced allowing for the use of RNAi to manipulate levels of specific genes. Furthermore, its high recombination rate makes the bee an excellent system for mapping quantitative trait loci (QTL) underlying complex behaviors. Using colonies of bees that were relatively sensitive or resistant to ethanol QTLs associated with ethanol sensitivity were analyzed (Ammons and Hunt 2008b). The honey bees used in this study were selected from 15 colonies with naturally mated queens. This study confirms that variation of sensitivity to ethanol exists within natural populations of insects just as observed for humans and rodents. Four QTLs were identified, and genes mapping to these regions encode proteins that have been found to be involved in ethanol sensitivity in other invertebrates and mammals including a dopamine receptor, and proteins involved in synaptic transmission, ethanol absorption and metabolism.

The real strength of the honey bee as a model system is the investigation of the effects of ethanol on learning and memory. In other invertebrates such as the fruit fly and the nematode work so far has mainly focused on the effects of ethanol on locomotion (Davies et al. 2004; Morgan and Sedensky 1995; Wolf et al. 2002 among others). However, addiction may share many pathways with learning and memory (Kreek et al. 2004; Nestler 2002) making the understanding of the effects of ethanol on learning and memory an important goal.

Honey bees quickly learn to associate olfactory or visual cues with sucrose rewards both in the field and in the controlled conditions in the laboratory (Menzel and Giurfa 2006). Bees to be used in laboratory experiments are commonly foraging adult worker bees captured at the entrance of the colony. Each bee is chilled and restrained in a small tube, leaving its antennae and proboscis free. The most commonly used learning assay is appetitive olfactory conditioning of the proboscis extension reflex during which bees learn to associate an odor puff (the conditioned stimulus, CS) with a sugar solution reward (the unconditioned stimulus, US) (Bitterman et al. 1983). Preliminary studies using appetitive olfactory conditioning suggested that consumption of ethanol by honey bees affected their ability to form an association between an odor and a sucrose reward (Abramson et al. 2000). A more detailed study showed that ingestion of an acute dose of ethanol before conditioning decreases acquisition in a dose dependent manner (Mustard et al. 2008). As observed in humans and rodents, the concentrations of ethanol required to affect learning were lower than those that had significant effects on locomotion, suggesting that ethanol may have distinct targets for learning versus motor function (Mustard et al. 2008; Maze et al. 2006). Furthermore, higher concentrations of

ethanol appear to affect olfactory processing, making it difficult for bees to distinguish odors they normally find to be quite distinct. If bees were fed ethanol solutions after conditioning had already taken place, their ability to recall the association was not compromised except at high ethanol concentrations (Mustard et al. 2008). This shows that in bees, as in humans and rodents, ethanol has a larger effect on learning than on the recall of information.

Further support for the influence of ethanol on learning come from the observation that ethanol influences the recall of a task learnt in the presence of alcohol. State dependency describes the phenomena that information that has been learned while the animal is under the influence of ethanol can only be recalled and used to solve a task when the animal is in the same state in which the information was learned, i.e. under the influence of ethanol. Olfactory adaptation in *C. elegans* is observed when animals previously exposed to an odor show a reduction in the response when the odor is offered again (Colbert and Bargmann 1995). During the acute phase of intoxication chemotaxis to a volatile odorant is slowed, but the animals can still move toward the odor source. When the animals are tested at the second exposure to the odor only, they do not show a reduction in attraction. However, in the presence of intoxicating ethanol concentrations and odor, they do show reduction. Thus, the task learnt in the presence of alcohol can only be recalled in the presence of the drug, establishing that *C. elegans* shows state dependent learning. Using this assay, it was shown that dopamine is involved in state dependent learning as mutants with defects in dopaminergic function do not show state dependency (Bettinger and McIntire 2004).

The honey bees also offers the ability to examine social behaviors and the advantage of having developed in a naturally complex environment as compelling reasons for using them as models for investigating the effects of ethanol on the nervous system. Recent work suggests that living in an enriched environment can significantly alter an individual's response to drugs of abuse (Laviola et al. 2008). Honey bees live in colonies containing thousands of individuals. Bees used in experiments are typically adults captured at the hive entrance rather than individuals raised in the controlled environment of the lab. Thus, they have experienced a complex environment in terms of both sensory information and social interactions throughout their development.

Finally the willingness of honey bees to consume ethanol might make them a good invertebrate model. Anyone who has sat outside with beer, wine, a margarita or a daiquiri will have experienced worker bees coming by for a sample. In fact, in a study examining the consumption preferences of honey bees, bees would willingly ingest a number of alcoholic beverages with Dekuyper Buttershots, Grenadine Cordial, Amaretto Di Amore and Wild Raspberry wine topping the list, while no bees would consume Old Charter Bourbon (Abramson et al. 2004a). The willingness of honey bees to consume ethanol containing solutions simplifies experiments in that the experimenter does not have to be concerned that treatment with ethanol is having secondary effects such as increased stress or aversive conditioning.

5 Comparison of Criteria Used to Classify Alcoholism and Behavioral Paradigms Used in Invertebrates

Animal models can be useful in analyzing two aspects of alcoholism. First, they can be used to gain understanding of the direct actions of ethanol on the organism and the roles of specific targets in predicting continued drug use. Second, analyses of behavioral patterns may also reveal behaviors that can be used as diagnostic indicators for the development of alcoholism. In humans low levels of response to ethanol correlate with the risk of becoming an alcoholic (Schuckit 1994). The increase in resistance to ethanol induced behaviors has been studied in flies, nematodes and honey bees, and a number of genes and mechanisms homologous to those in humans have been identified (see section above). Here, we focus on the direct comparison of behaviors associated with alcoholism and alcohol abuse in humans and invertebrates. Alcohol use and dependence of a person is normally evaluated by the diagnostic criteria of the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). A comparison of the DSM-IV guidelines and behaviors to studies in invertebrate models are summarized in Table 1. However, it is still open to debate as to which of the behaviors listed in Table 1 are actually causal for drug abuse and alcoholism. Ideally the behavior of animal models should mimic different aspects of these criteria, so that knowledge gained from models can be used for the development of pharmacological or behavioral treatments of alcoholism in humans. For example, Piazza and colleagues have developed assays using the self administration of cocaine in rats that closely correspond to the criteria for addiction in humans (Deroche-Gamonet et al. 2004; Kasanetz et al. 2010). These three criteria are: (1) the individual has difficulty stopping drug use or limiting drug intake, (2) the subject has extremely high motivation to take the drug with its activities focused on drug procurement and consumption and (3) substance use is continued despite harmful consequences. Although similar assays for criteria 1 and 2 have not been developed for examining ethanol addiction in invertebrates, an assay for criteria 3 has recently been developed for *Drosophila* (Devineni and Heberlein 2009). When quinine, a compound that usually is aversive for flies, is added to ethanol containing food flies that have been drinking ethanol for the previous five days will preferentially consume quinine–ethanol–food over food without ethanol or quinine. Thus, flies will continue to consume ethanol despite negative consequences.

5.1 Tolerance

The DSM-IV guidelines include the development of high levels of tolerance as one of its criteria. As described above flies develop both rapid and chronic tolerance to the effect of ethanol on postural control, the decrease in locomotor activity,

Table 1 A comparison of the DSM-IV guidelines and behaviors to studies in invertebrate models

Diagnostic criteria in humans	Animal model	Invertebrate model	References
1. Tolerance- increase of ethanol doses required for intoxication	Rapid and chronic tolerance	*D.melanogaster*	Scholz et al. (2000); Berger et al. (2004); Urizar et al. (2007); Li et al. (2008); Cowmeadow et al. (2005)
2. Withdrawal symptoms- alleviated by alcohol	Withdrawal- like syndroms	*C.elagans*	Mitchell et al. (2010)
3. Alcohol is consumed in greater quantities	Chronic drinking leads to increase	*D.melanogaster*	Devineni and Heberlein (2009)
4. In-ability to control alcohol use	Reinstatement of ethanol consumption	*D.melanogaster*	Devineni and Heberlein (2009)
5. Excessive amount of time spent with alcohol use and recovery	Alcohol self-administration (with high work load)	*D.melanogaster, A.mellifera*	Devineni and Heberlein (2009); Abramson et al. (2004a, b); Maze et al. (2006)
6. Reduction of important activities not related to alcohol	Choice paradigms	*D.melanogaster*	Devineni and Heberlein (2009)
7. Alcohol use despite known alcohol problems	testable?		

sedation, and the recovery from sedation (Scholz et al. 2000; Berger et al. 2004; Cowmeadow et al. 2005; Urizar et al. 2007; Li et al. 2008).

Genes involved in ethanol tolerance have been isolated and characterized. For example, the analysis of the *hangover* mutant—a mutant with reduced tolerance—uncovers a cellular stress response involved in mediating ethanol tolerance. This stress response is similar to a response to heat shock stress (Scholz et al. 2005). The novelty of this finding is that ethanol can cause a cellular stress response leading in turn to an alteration in behavior. In humans, a Hangover related protein is associated with alcoholism and post mortem brains of alcoholics show reduced levels of the protein (Riley et al. 2006). To better understand the molecular basis of the cellular stress response that alters ethanol induced behavior, it is important to dissect the function of Hangover protein in more detail. A link between cellular stress responses and changes in ethanol induced behaviors were further supported by the finding in *C. elegans* that the expression of the heat shock protein 16 (Hsp16), a protein that is induced after exposure to environmental stress, is increased after ethanol exposure (Thompson and de Pomerai 2005). Additional *Drosophila* mutants uncovering functional similarities to vertebrates on a cellular level with defects in ethanol tolerance

have been identified. Mutants of the *jwa* gene, a homolog of the mouse *addicsin* gene, also show reduced tolerance to the effect of ethanol on postural control (Li et al. 2008). In mice the *addicsin* gene is implicated in the development of morphine tolerance and dependence (Ikemoto et al. 2002). These findings suggest that the mechanisms by which the brain responds during the development of tolerance to ethanol on a cellular level might be conserved between humans and invertebrates.

5.2 Withdrawal

A paradigm that measures a withdrawal-like syndrome has been developed in *C. elegans* (Mitchell et al. 2010). A food race assay in which worms move towards a bacteria source presented on an agar plate is used to quantify the effect of increasing ethanol concentrations on foraging behavior. In the presence of increasing ethanol concentrations, the number of worms that reach the food source declines. After a prolonged pre-exposure to ethanol followed by a recovery period, a reduced number of worms reach the food source indicating that the nematodes have impaired movement even in the absence of ethanol. In addition, the nematodes show an increase in the number of omega turns that occur independent of reversals. When small amounts of ethanol are added back to the worms after the recovery period, the locomotor performance once again increases suggesting that the worms indeed were suffering from withdrawal. This withdrawal-like behavior requires normal processing of neuropeptide function since *egl-3* mutants, which have defects in a pro-protein Convertase required for processing of neuropeptides, do not show withdrawal symptoms (Mitchell et al. 2010).

5.3 Chronic Increases in the Consumption of Alcohol Over Time, Inability to Control Alcohol Use, and Excessive Amounts of Time Spent with Alcohol

The willingness of honey bees to consume ethanol solutions has not yet been extended to chronic exposure to ethanol. However, the voluntary ingestion of ethanol by fruit flies shows that over time they increase their preference for consuming ethanol containing food. For this analysis, food intake is measured with an assay analogous to a two-bottle choice assay, except using capillary feeders (CAFE) (Ja et al. 2007). Flies are offered the choice between feeding from a capillary containing food alone or a capillary with food and ethanol. The amount of food consumed from each capillary is determined and a preference index for the consumption of food containing ethanol is calculated. Flies will voluntarily consume levels of ethanol that lead to measurable internal ethanol concentrations

(Devineni and Heberlein 2009). The inability of animals to control alcohol use can also be tested using a reinstatement of ethanol consumption paradigm (Spanagel et al. 1996; for review Bell et al. 2006 among others). Intriguingly, after a phase of deprivation, flies reinstate their intake of ethanol-containing food. Furthermore, they also consume ethanol-containing food when normally aversive substances are added, suggesting that flies are overcoming negative effects to obtain intoxicating amounts of ethanol (Devineni and Heberlein 2009). This observed behavior is consistent with previous choice experiments where adding quinine as an aversive stimulus to the ethanol solution did not alter the alcohol deprivation effect in rats intermittently allowed to consume ethanol for eight months (Spanagel et al. 1996).

5.4 Other Behaviors Associated with Alcoholism

When comparing animal models to these diagnostic criteria, one criterion that cannot be tested directly in animals is the continued use of alcohol despite the knowledge of persistent problems associated with alcohol use. However, binge intake following alcohol deprivation is considered to be comparable (Bennett et al. 2006). Remarkably some animal models such as the alcohol preferring rats (P rats) fulfill the different criteria associated with alcoholism and the phenotypes resemble those seen in humans misusing alcohol (Bell et al. 2006). In vertebrate models the excessive amount of time spent with alcohol including obtaining alcohol, drinking and recovery are measured in various paradigms. For example under limited access, rats will self-administer ethanol until intoxicated. The maintenance of ethanol preference while other nutritive palatable solutions are present is another indication of an excessive amount of time spent with alcohol (Bell et al. 2006). The two feeder choice paradigm for flies might reflect this behavior in part and could potentially be extended with an additional source of nutritious solution (Devineni and Heberlein 2009).

The influence of ethanol on social behavior can also be analyzed in flies and honey bees. Flies of both sexes interact during courtship. *Drosophila* males normally court female flies. Upon repeated ethanol exposure these behavior change and male flies start to court other males. This change in behavior is dose-dependent as increasing concentrations of ethanol lead to increase in courtship behavior, although mating success declines. The male/male courting behavior depends on dopamine neurotransmission since inhibition of dopamine signaling reduces this behavior (Lee et al. 2008). Honey bees show intricate social behaviors, since they live in a highly social environment. Several well characterized social interactions such as food sharing, social grooming, colony defense and inter-bee communication via dancing can be used to examine ethanol's effects on social interactions. Several studies have examined the relationship between ethanol consumption and aggressive bee behavior as assayed by the number of times a black patch placed in front of a colony was stung. Africanized honey bees foraging on ethanol solutions

behaved more aggressively compared to when they consumed sucrose alone (Abramson et al. 2004b). In addition, comparisons between bees from two different colonies of European honey bees showed that those from a more defensive (or aggressive) colony were more sensitive to the effects of ethanol compared to bees from a less defensive colony (Ammons and Hunt 2008b).

These initial studies suggest that as well as providing information on the genetic and neural mechanisms underlying the actions of ethanol, *Drosophila* and honey bees are suitable models for studying the effects of ethanol on social behaviors. However, there are still questions to be answered. For example, how can the observed behaviors in the different species be compared, what are the causal relationships between the different behaviors observed, and what are the underlying mechanisms causing the behavioral changes?

6 Conclusions

The results reviewed above indicate that (1) in invertebrates ethanol is metabolized by similar mechanisms as in vertebrates (2) invertebrates show similar signs of intoxication (3) molecular pathways mediating the actions of ethanol and/or alcoholism related behaviors are similar between vertebrates and invertebrates and (4) behaviors that are used as criteria to determine alcoholism such as ethanol tolerance, preference for ethanol consumption, and reinstatement of ethanol use after periods on ethanol withdrawal can be analyzed in invertebrates. Taken together, these results suggest, that invertebrate models can be effectively used for the discovery of new molecules mediating the direct action of ethanol, to identify genes involved in the genetic predisposition for alcohol abuse/alcoholism, and to identify genes and mechanisms involved in alcoholism related behaviors. Invertebrate models might be further used for screening potential therapeutic agents for treating alcoholism. However, invertebrate models also have some limitations. Molecules in invertebrates are similar, but not identical. The shorter life spans of the invertebrates might interfere with long term studies of alcohol treatments. Although the continuation of alcohol consumption despite the knowledge of alcohol related problems observed in alcoholics cannot be tested in invertebrates, this is a general problem shared with other rodent model systems. Invertebrates offer many possibilities for advancing our understanding of the genes and mechanisms underlying alcohol induced behaviors. In addition, the elegant genetic tools available in invertebrate models can be used to dissect causalities between different behavioral components and their contributions to the development and maintenance of alcoholism.

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Part IV
Novel Insights from Brain Imaging

The Dopamine System in Mediating Alcohol Effects in Humans

K. Charlet, A. Beck and A. Heinz

Abstract Recent brain–imaging studies revealed that the development and maintenance of alcohol dependence is determined by a complex interaction of different neurotransmitter systems and multiple psychological factors. In this context, the dopaminergic reinforcement system appears to be of fundamental importance. We focus on the excitatory and depressant effects of acute versus chronic alcohol intake and its impact on dopaminergic neurotransmission. Furthermore, we describe alterations in dopaminergic neurotransmission as associated with symptoms of alcohol dependence. We specifically focus on neuroadaptations to chronic alcohol consumption and their effect on central processing of alcohol-associated and reward-related stimuli. Dysfunctional reward processing, impaired reinforcement learning and increased salience attribution to alcohol-associated stimuli enable alcohol cues to drive alcohol seeking and consumption. Finally, we will discuss how the neurobiological and neurochemical mechanisms of alcohol-associated alterations in reward processing and learning can interact with personality traits, cognition and emotion processing.

Keywords Dopamine · Acute alcohol effects · Chronic alcohol effects · Imaging · Cue-reactivity · Reward system

List of Abbreviations

5-HT	Serotonin
ACC	Anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors
BAC	Blood alcohol concentration

K. Charlet (✉) · A. Beck · A. Heinz
Department of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin, Chariteplatz 1, Berlin, Germany
e-mail: katrin.charlet@charite.de

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BOLD	Blood oxygen level dependent response
CNS	Central nervous system
CS	Pavlovian conditioned stimulus
DA	Dopamine
DLPFC	Dorsolateral prefrontal cortex
DRD1	Dopamine D1 receptor
DRD2	Dopamine D2 receptor
DRD3	Dopamine D3 receptor
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EtOH	Ethanol
fMRI	Functional magnetic resonance imaging
F-DOPA	[18F]Fluoro-L-dopa
GABA	Gamma-aminobutyric acid
HA	Harm Avoidance
IAPS	International Affective Picture System
LTP	Long-term potentiation
MPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
NMDA	N-methyl-D-aspartate
NS	Novelty Seeking
OFC	Orbitofrontal cortex
PET	Positron emission tomography
PFC	Prefrontal cortex
SPECT	Single photon emission computed tomography
SPM	Statistical Parametric Mapping
SSRI	Selective serotonin reuptake inhibitors
T1	T1-Weighted magnetic resonance sequence
TPQ	Cloningers Tridimensional Personality Questionnaire (Cloninger 1987b)
VTA	Ventral tegmental area
WM	Working memory

Contents

1 Introduction 463
1.1 Acute Alcohol Effects on Dopaminergic Neurotransmission 464
1.2 Excitatory and Depressant Acute Alcohol Effects on Dopamine and Related Neurotransmitter Systems and Behavior 466
1.3 Effects of Chronic Alcohol Intake on Dopamine Neurotransmission 472
2 Summary 480
References 480

1 Introduction

It has been well described that an increase in dopamine release is prominent in the rewarding and positive reinforcing effects of drugs of abuse (Heinz 2000, 2009; Koob 1992; Tapert et al. 2004; Wise and Rompre 1989). Olds and Milner (1954) showed that rats, which accidently had electrodes implemented into their septum instead of the formatio reticularis, would excessively stimulate their basolimbic area until complete exhaustion and even ignore food (Olds and Milner 1954). In 1963, Heath described two human patients under self-stimulation treatment, which stimulated regions of the brain at a high frequency (Bishop et al. 1963; Heath 1963). Since, the activated neurocircuits included dopaminergic projections, these findings initiated investigations of the **dopaminergic reward system**, which explored how dopamine-associated reinforcement establishes persisting habits (Birbaumer and Schmidt 2003; Wise 2002).

Intensive research in the past decades identified various neurotransmitter systems participating in the development and maintenance of increased and chronic alcohol intake in humans, e.g., dopaminergic (DA), serotonergic (5-HT), opioidergic and glutamatergic neurotransmission (Heinz et al. 2008, 2009; Mann 2004; Oscar-Berman and Bowirrat 2005). The mesocorticolimbic DA circuitry emerged to be of central importance, since alcohol and other drugs of abuse release DA in the striatum, which promote drug- seeking behavior, and consecutive intake. In comparison to primary or **neutral reinforcers** (like food, sleep, sex, or money), the effect of drugs on DA release does not appear to habituate (Di Chiara and Bassareo 2007). Presumably, this is caused by the drugs' pharmacological activation of dopaminergic stimulation compared to primary rewards necessary for survival (Wise and Rompre 1989). It is, therefore, assumed that addictive drugs "hijack" the reward system, which preferentially responds to drug-associated reinforcement at the expense of non-drug reward (Gardner 2005).

In 2009, 9.5 million Germans consumed alcohol in a health-risking manner and 1.3 million subjects were considered to be alcohol-dependent (Drogenbeauftragte der Bundesregierung 2009). To address alcohol-specific processes, which contribute to the development of alcohol dependence, chronic alcohol effects on the human body and the brain need to be assessed and distinguished from effects associated with acute and intermittent alcohol use.

The major components of the **dopaminergic mesocorticolimbic circuit** consist of dopaminergic projections from the **ventral tegmental area (VTA)** and substantia nigra to the **ventral striatum** including the **nucleus accumbens (NAc)**, the amygdala, olfactory tubercle and frontal and limbic cortices (for an extensive review see Ikemoto 2007). Key limbic projections to the NAc include inputs from the (pre)frontal cortex, amygdala and hippocampus. Thus, limbic information received by the NAc can be projected to neurocircuits contributing to motivated behavior via pallido-thalamic and thalamo-cortical projections (Koob 1992); (see Fig. 1). The integrity of these specific pathways is crucial to provide an adequate response to internal and external stimuli and to govern attention and intentions

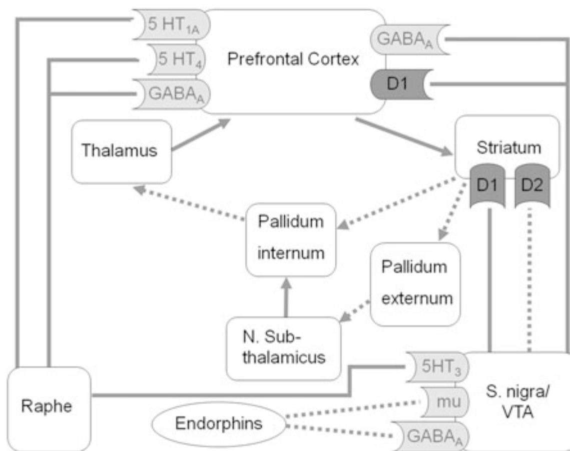

Fig. 1 Interaction of primary neurotransmitter systems involved in the acute initiation of alcohol intake. Inhibitory effects [via D_2 receptors (DRD_2) on the target cells (e.g., in the striatum) mediating GABAergic neurotransmission] are symbolized by dotted lines and excitatory effects [via D_1 receptors (DRD_1) on the target cells (e.g. in the striatum) mediating glutamatergic neurotransmission] by solid lines (modified according to (Heinz et al. 2009)). Abbreviations: DRD_1 = dopamine D_1 receptor; DRD_2 = dopamine D_2 receptor; GABA = gamma-aminobutyric acid

related to a particular stimulus. The NAc consists of two distinguishable regions: the core and the shell, which are innervated by DA neurons arising from the VTA (Di Chiara 2002; Uhart and Wand 2008). While the ventral striatum appears to be involved in motivated behavior and the attribution of **incentive salience** to novel, reward-associated cues, the **dorsal striatum** plays a major role in **habit formation** (Robinson and Berridge 1993).

1.1 Acute Alcohol Effects on Dopaminergic Neurotransmission

During alcohol consumption, alcohol passes through the oesophagus into the stomach. There, about 20% of the consumed alcohol is absorbed by the gastric mucosa and delivered directly into the bloodstream. The remaining 80% enter blood circulation through the small intestine mucosa membrane. Through this particular pathway, ethanol (EtOH) is distributed throughout the human body within seconds and overcomes the blood–brain barrier due to EtOH liposoluble properties (Lindenmeyer 2001). In the brain, several neurotransmitter systems interact in a complex manner (Fig. 1): It has been shown that both the opioidergic and the DA neurotransmitter systems are involved in the rewarding effects caused by EtOH. It has been originally hypothesized by Wise (1982) that drug and alcohol-induced DA release mediates the hedonic feeling associated with drug-induced reward (Di Chiara 2002; Wise 1982, 1996). However, this hypothesis has

not been supported via pharmacological blockage of DA neurotransmission. The results of DA blockage studies in both animals and humans resulted in (motivational) apathy rather than anhedonia. Therefore, DA has been attributed a role in response-eliciting, but not in hedonic properties ("wanting" instead of "liking") (Boileau et al. 2003; Di Chiara 2002; Heinz et al. 2009, 1998).

Robinson and Berridge (1993) suggested that **"liking"** refers to the experience of pleasure, which is controlled by opioid and also potentially endocannabinoid and gamma-aminobutyric acid (GABA)-benzodiazepine neurotransmitter systems. One hedonic hotspot for opioidergic enhancement was located in the NAc (Berridge and Kringelbach 2008; Berridge et al. 2009; Pecina and Berridge 2005; Robinson and Berridge 1993). Animal and human studies showed that alcohol stimulates endorphins, which act on mu-opiate receptors in the NAc and also stimulate DA release in the same brain area via indirect effects on GABAergic neurons (de Waele et al. 1994; Ramchandani et al. 2010; Spanagel et al. 1992). In detoxified alcohol-dependent patients, an increase of mu-opiate receptors can be found in the ventral striatum and medial prefrontal cortex (PFC), which was correlated to alcohol craving (Heinz et al. 2005a).

The other component of reward, ***"wanting"*** or "*craving*", has been associated with the attribution of incentive salience to the drug of abuse and to drug-associated stimuli. Thus results in *a type of incentive motivation that promotes direct approach toward reward-related stimuli and consumption of rewards* and does not require elaborate cognitive expectations (Berridge et al. 2009). *Wanting* is assumed to be correlated with (phasic) cue-dependent DA release in the ventral striatum. Here, the work of Schultz et al. (1997) is of fundamental importance, which elucidated the role of DA neurons in mediating reward processing and **reward-dependent learning**. In a crucial experiment, monkeys received oral administration of an unexpected salient reward,.i.e., fruit juice, which elicited immediate DA-bursts in VTA dopaminergic neurons (Schultz et al. 1997). Schultz et al. (1997) showed that these phasic activations are not specific to different types of rewarding stimuli; DA neurons react similarly to different kinds of appetitive stimuli and are also activated by novel stimuli that elicit orientating behavior. Phasic changes in the DA discharge can also be seen when reward is predicted via a Pavlovian conditioned stimulus (CS): bursts of dopaminergic firing followed directly after the presentation of a CS, which predicted upcoming reward. On the other hand, neural DA responses fail to appear at the moment of a fully predicted reward-experience because they appear only if a **prediction error** occurs; i.e., if the received reward is larger than expected. If a predicted reward is not received, DA neurons firing is reduced (Schultz et al. 1997). Therefore, the DA neurotransmitter system functions as a signalling network registering the occurrence of salient stimuli and the unexpected absence of reward, i.e., so-called prediction errors. However, DA in the NAc does not only code the salience, it also reflects the value of a potential reinforcer (Tobler et al. 2005). It has been suggested that reward-associated, stimulus-dependent DA release may be specifically vulnerable to **sensitization**, i.e., a stronger neuronal and behavioral response upon re-exposure to the pharmacological effects of repeated administration of dopaminergic

drugs (Heinz et al. 2004a; Robinson and Berridge 1993). Altogether DA enhancing and endorphin-stimulating effects of **acute alcohol intake** can promote both the described hedonic response and a motivational response and may facilitate learning of motivational reactions to drugs and drug-associated stimuli.

In drug-susceptible individuals, neural sensitization of incentive salience by drugs of abuse like alcohol may result in compulsive “wanting”, which leads to consecutive drug intake. This can happen regardless of whether or not the drugs are “liked”, and thus contribute to the development of addiction (Berridge et al. 2009).

Nevertheless, DA involvement in incentive salience also affects processing of aversive stimuli. In the NAc, DA and glutamate interactions have been associated with fearful experiences common in both appetitive and fearful behaviors. That points to multiple functional modes of these substrates, depending on specific external and internal factors (Berridge et al. 2009). In humans, DA synthesis capacity in the amygdala was closely associated with processing of aversive stimuli (Kienast et al. 2008).

A NAc-VTA-NAc-circuitry has been described by Adermark and colleagues (2010), which can be activated by drugs of abuse like EtOH through inhibitory GABAergic medium spiny neurons. These neurons project from the NAc to the VTA and tonically regulate DA firing (Adermark et al. 2010). Glycine receptors in the NAc appears to control baseline DA levels by mediating the DA-elevating and -reinforcing effects of EtOH (Molander et al. 2005; Söderpalm et al. 2009). In accordance, Vengeliene et al. (2010) demonstrated that administration of a selective blocker of glycine transporter 1 caused a persisting reduction of compulsive relapse-like drinking without the development of tolerance for a at least 6 weeks treatment-free period. These new findings of Adermark et al. (2010) support the idea that extracellular DA and taurine levels are interconnected, and suggest that an elevation in extracellular taurine concentrations might be required in order for EtOH to enhance DA levels in the NAc.

Complementary effects of EtOH have been documented in the past: while DA (and noradrenergic) mechanisms, along with the **endogenous opioid systems** of the brain, seem to be implicated in the rewarding effects of EtOH via activation of positive reinforcement pathways, the 5-HT system seems to be associated with the mediation of negative reinforcement (Heinz et al. 2001; Nevo and Hamon 1995; Valenzuela 1997) and 5-HT dysfunction is also associated with anxiety and depression in alcoholism (Heinz et al. 1998).

1.2 Excitatory and Depressant Acute Alcohol Effects on Dopamine and Related Neurotransmitter Systems and Behavior

1.2.1 Excitatory Effects of Alcohol

EtOH has different pharmacological effects on the central nervous system (CNS). In humans, an excitatory effect occurs before the depressant properties ensue with

further ingestion of alcohol. It was shown with low doses of alcohol, that one can observe physiological excitation, which is correlated with improved performance on motor, cognitive and information-processing tests (Pohorecky 1977).

Findings from animal self-stimulation studies using doses of EtOH between 0.2 g and 2 g/kg suggest an activation of DA neurons and other catecholamines (Carlson and Lydic 1976; Ollat et al. 1988; Pohorecky 1977). Investigations using more specific synthetic DA agonists and antagonists have revealed that **DA D_1 receptor (DRD_1)** and **DA D_2/D_3 receptors (DRD_2/DRD_3)** are implicated in the increased EtOH-induced locomotor activity at lower doses (Cohen et al. 1997).

In human studies, alcohol (1 ml/kg) versus orange juice intake induced a significant reduction in binding of the DA receptor ligand [^{11}C]raclopride to DRD_2/DRD_3 in the ventral striatum/NAc, which is indicative of increased extracellular DA levels that compete with the ligand for receptor binding. In this **positron emission tomography (PET)** study, the magnitude of change in [^{11}C]raclopride binding correlated with alcohol-induced increases in heart rate, a marker of the **psychostimulant effects** of the drug and with the personality dimension of impulsiveness (Boileau et al. 2003).

In an experiment to assess the conscious experience of EtOH-stimulating effects, Williams (1966) asked volunteers to rate anxiety and depression at prearranged cocktail parties. It was observed that negative affective states decreased significantly with low levels of EtOH consumption (Williams 1966). Other behavioral studies reported increased talkativeness, feelings of elation and happiness, euphoria, relaxation and stress-reducing, anxiolytic effects as a result of alcohol intake (Ekman et al. 1963, 1964; Gilman et al. 2008).

Additional studies found that subjects expect alcohol to increase their sociability. After consuming EtOH, individuals reported positive feelings like being "alert", "quick-witted" and "attentive". The amount of alcohol intake correlated positively with the alcohol expectancy factor termed "sociability" (Duka et al. 1998). Further, sociability-related alcohol expectancies were associated with sociability-related self-concept ratings when participants were exposed to alcohol primes (i.e., pictures or words associated with alcohol), but not when participants were exposed to neutral primes (Hicks et al. 2009). Individuals who experience greater stimulant-like effects from an acute alcohol dose reported greater drug-liking and elation, as well as greater behavioral preference for EtOH (over placebo) compared to individuals who experience mostly sedative-like effects of EtOH (de Wit et al. 1989, 1987).

In a study by Holdstock et al. (2000), healthy subjects consumed either a beverage with ethanol (0.2, 0.4, or 0.8 g/kg) or a placebo in a randomized and double-blinded conditioned manner for a total of four laboratory sessions. Subjects who were classified as habitual, moderate or heavy EtOH users (consumption of $\geq$8 drinks/week with frequent binge episodes) displayed greater stimulant-like effects of alcohol. This is consistent with the idea of Newlin and Thomson (1990, 1999), who suggested that individuals who experience greater stimulant-like effects during the ascending limb and reduced sedative-like effects on the descending limb of **blood alcohol concentration (BAC) curve** may be at greater

risk for an increased alcohol intake and alcohol-associated problems (Holdstock et al. 2000; Newlin and Thomson 1990; 1999). Accordingly, alcoholics given a low dose of alcohol reported more stimulant effects than social drinkers, and individuals with a positive **family history of alcoholism** reported a greater initial response to alcohol challenge compared to subjects with a negative family history of alcohol addiction (Crabbe et al. 2010). The issue of (ethanol) reward in addiction and how to measure drug reward sensitivity (e.g., effects of drugs) in humans and animals has been critically reviewed recently by Stephens et al. (2010).

These stimulating effects of EtOH are thought to have a direct effect on DA neurons (Carlsson et al. 1974) and as discussed before, may primarily contribute to the motivation for further drug intake. The hedonic effects of alcohol, on the other hand, have been associated with the activation of mu-opiate receptors and can be blocked by naltrexone (Volpicelli et al. 1995).

The stimulating effect of EtOH appears to be modulated by the interaction of DA and 5-HT neurotransmission. 5-HT function is affected by alcohol and influences the mesolimbic DA reward system (Heinz et al. 2004a, 2001; LeMarquand et al. 1994a, b; van Erp and Miczek 2007). For example, acute EtOH can facilitate 5-HT reuptake in the hippocampus and decrease 5-HT_{1A} receptor functioning in the cortex (LeMarquand et al. 1994a). 5-HT uptake inhibitors, i.e., zimeldine, citalopram, viqualine and fluoxetine, have been shown to decrease alcohol consumption in male subjects who are classified as moderate social drinkers (Gorelick 1989; LeMarquand et al. 1994b). However, these observed effects were of transient nature and data from studies of **selective serotonin reuptake inhibitors (SSRIs)** in alcohol dependence are heterogeneous (see review by Kiefer and Mann, 2005). A meta-analyis by Garbutt et al. (1999) does not support SSRIs in the treatment of alcohol addiction.

Nevertheless, **5-HT dysfunction** was associated with some behavior patterns predisposing to for example impulsive **aggression**, negative mood states, and a low response to alcohol intake (Heinz et al. 2001). Furthermore, chronic alcohol intake may exert neurotoxic effects on the 5-HT system. Depending on an individual's vulnerability, these neurotoxic effects may result in loss of central 5-HT function and give rise to negative mood states, such as anxiety and depression (Heinz et al. 1998).

As the World Health Organization (2007) stated, alcohol, compared to all other psychoactive substances, is arguably the most potent agent for eliciting aggression and reducing behavioral control (WHO 2007). A meta-analysis by Bushman and Cooper (1990) revealed that acute alcohol consumption does indeed facilitate aggressive behavior (Bushman and Cooper 1990). 5-HT modulates aggressive behavior via its effects on negative mood states in interaction with other neurotransmitters, of which corticolimbic DA continues to be of interest for its critical role in integrating motivational and motor functions (Heinz et al. 2003, 2001; Knutson et al. 1998; Robbins et al. 1989). It was shown by Ase et al. (2000) that the main 5-HT influence on accumbal DA neurons originates in the dorsal raphe nucleus (Ase et al. 2000), which has also been implicated in the regulation of

alcohol self-administration (Yoshimoto and McBride 1992). As a result of these observations, animal experiments investigated the activity of accumbal DA and 5-HT during the phases of initiation, execution, and termination of alcohol drinking and observed interactions between DA activation and aggressive behavior (van Erp and Miczek 2007).

In human behavioral experiments, such as the Taylor aggression paradigm, where electric shocks are received from and administered to a fictitious opponent during a competitive task, acute alcohol intoxication in male social drinkers increases aggressive behavior at a BAC of 0.08% (Giancola and Zeichner 1997). Hereby it is assumed that an amygdala-mediated differentiation between threatening and non-threatening stimuli is disrupted during acute alcohol intoxication. Thus, the amygdala and other related neuronal networks may less likely to correctly identify threatening stimuli. This may trigger an increase in approach and aggression in some individuals (Gilman et al. 2008). It was suggested that negative mood states contribute to aggressive behavior by facilitating limbic (amygdala) processing of aversive and threatening stimuli in serotonin-reduced neuronal states (Heinz et al. 2001).

1.2.2 Depressant Effects of Alcohol

Besides the excitatory effect of alcohol, EtOH can also act pharmacologically as a depressant of neuronal activity. As the dose of alcohol increases, **sedative effects** should become greater than stimulatory effects (Martin et al. 1993). Thus, when blood alcohol levels are declining (i.e., the descending limb of intoxication), alcohol's effects are largely sedative and unpleasant (Ray et al. 2009). These unpleasant feelings can be subjectively experienced as a hangover, exhaustion and depression, or may even cause vomiting (Nagoshi and Wilson 1989).

There is broad individual variability in the phenomenology of EtOH response (de Wit et al. 1989; Duka et al. 1998; Holdstock et al. 2000), and, stronger subjective experiences of the sedative and unpleasant effects of alcohol have been associated with decreased alcohol consumption (Ray et al. 2009; Schuckit and Smith 1996). On the other side, individuals with a family history positive for alcoholism displayed less intense response to the aversive aspects of acute alcohol consumption, which is assumed to be predictive of alcohol problems in later life. These specific individuals also demonstrated lower hormonal responses (lower cortisol, prolactin and adrenocorticotropic hormon levels) after alcohol drinking (see review by Crabbe et al. 2010).

In a non-human behavioral experiment by Carlson and Lydic (1976), higher doses of EtOH suppressed the medial forebrain bundle reward system, which is known to function mainly via dopaminergic neurotransmission (Carlson and Lydic 1976). Besides inhibiting DA neurotransmission, alcohol also interacts with GABAergic, glutamatergic and opioidergic neurotransmitter systems, which may all contribute to the sedative effects of EtOH (Cohen et al. 1997).

The depressant actions of EtOH on the CNS have indeed been related to facilitation of GABA neurotransmission via alcohol effects on the benzodiazepine/ GABA receptor complex (Hunt 1983; Ticku 1989; Wang et al. 2000). Furthermore, Lovinger et al. (1989) demonstrated alcohol's dose-dependent inhibition of neuronal activation induced by the glutamate agonist *N*-methyl-D-aspartate (NMDA) in hippocampal neurons. Further studies supported the hypothesis that EtOH disrupts **glutamatergic neurotransmission** by decreasing cationic conductance through the NMDA receptor and thus inhibits NMDA receptor responses (Nevo and Hamon 1995; Tsai et al. 1995).

In addition, there is an EtOH-induced effect of analgesia, which is mediated by the opioidergic system. This effect was demonstrated in animal study, which investigated the ability of EtOH to stimulate opiate receptors; administration of EtOH (2–3.5 g/kg) in rats induced an analgesic effect (Jørgensen and Hole 1981).

In vivo, Volkow and associates (1990) observed in a PET study of human subjects that EtOH inhibits cortical and cerebellar glucose metabolism, supporting similar findings in animal studies. These inhibiting effects of alcohol on regional brain metabolism were shown to be larger in alcohol-dependent patients than in healthy subjects after alcohol-administration. The authors assumed that this decrease of energy metabolism is due to the EtOH action itself (Volkow et al. 1990).

1.2.3 Acute Alcohol Effects on Cognition

At BAC levels lower than the legal intoxication limit, i.e., 0.02–0.03%, mental function impairments in attention and vigilance can be detected (Koelega 1995). Further studies using **electroencephalography (EEG)** demonstrated that alcohol intoxication disrupts neurophysiological indices of stimulus processing in attentional (Grillon et al. 1995; Jääskeläinen et al. 1999; Marinkovic et al. 2001), semantic (Marinkovic et al. 2004) and memory domains (Valenzuela 1997). Regarding psychomotor control, a moderate dose of alcohol (0.65 g/kg) impairs cognitively demanding psychomotor tasks such as inhibiting responses in a go/no-go task (Fillmore et al. 2005). In general, sensitivity to the impairing effects of alcohol is relative to the complexity of the psychomotor task, therefore, more demanding tasks may be hindered by alcohol at lower levels than easier tasks [e.g., Hindmarch et al. (1991)]. In addition, alcohol affects cognitive processes such as judgment. Moderate doses of alcohol (0.5–0.8 g/kg) have been shown to lead to an overoptimistic assessment of a person's own ability (Tiplady et al. 2004), and to reduced perceptions of risk, thus contributing to risk-taking behavior by altering expectations about negative consequences (Fromme et al. 1997). Altogether, these studies demonstrate that moderate to heavy alcohol consumption can affect cognitive processes such as judgment, reasoning and decision making (Brumback et al. 2007).

Further studies showed that acute intoxication results in a disproportionate impairment of **executive functions** such as planning, working memory (WM) or complex behavioral control (Peterson et al. 1990). DA projections to the PFC regulate WM function (Egan et al. 2001; Goldman-Rakic 1995) and prefrontal DA

innervations modulate fronto-subcortical circuits, which regulate striatal DA release (Heinz 2000). Primate **conditioning experiments** by Williams und Goldman-Rakic (1995) directly showed that firing of PFC neurons sustains WM information. Thereby, the activity of these PFC neurons depends on optimal DA stimulation, which is reflected in an "inverted U-shaped curve" of DA effects on PFC WM functions (Williams and Goldman-Rakic 1995). As human studies indicated, the administration of DRD_2 agonists also improved WM performance, whereas the blockage of DRD_2 diminished WM functions (Luciana et al. 1992; Williams and Goldman-Rakic 1995).

PFC dysfunction can contribute to behavioral disinhibition and compulsive drug intake (Lubman et al. 2004). Two relevant prospective **functional magnetic resonance imaging (fMRI)** studies showed that PFC dysfunction is associated with the subsequent relapse risk in addicted patients: (1) Grüsser et al. (2004) observed that increased activation of the cingulate and adjacent medial PFC (and the striatum) elicited by alcohol-associated cues predicted relapse in alcohol-dependent patients; and (2) Paulus et al. (2005) described that a decreased activation of the PFC during decision making was associated with the subsequent relapse risk in methamphetamine-dependent subjects (Grüsser et al. 2004; Paulus et al. 2005). However, to date the role of DA neurotransmission in these functional alterations remains to be explored.

1.2.4 Acute Alcohol Effects on Emotion Regulation

Mood changes have been observed as a result of alcohol's stimulating and depressant effects. In a fMRI-study, Gilman et al. (2008) investigated the brain response to alcohol intoxication and emotional stimuli. The authors observed that an increased response to fearful faces in the placebo condition (intravenous saline infusion) was abolished in the alcohol condition (intravenous alcohol infusion with a maximal BAC of 0.08%). Thus, alcohol may affect **emotional processing** in limbic and visual regions by decreasing the difference in activation between threatening and non-threatening stimuli, which can contribute to both the anxiolytic properties of alcohol and to risky decision making during intoxication. The authors also observed a substantial activation of the striatum across emotional conditions in the alcohol versus placebo condition. As Gilman et al. (2008) show, this increase in activation can be modulated by negative emotional stimuli: the participants exhibited decreased striatal activation when viewing fearful faces, a finding which suggests that threatening stimuli may have attenuated the reinforcing effects of alcohol in the striatum.

1.2.5 Acute Alcohol Effects on Personality

Acute alcohol intake can increase impulsive behavior (Dougherty et al. 2008; Marczinski et al. 2007). However, **impulsiveness** is a multi-faceted construct,

which can occur in several domains including motor (inability to inhibit behavioral responses), cognitive (impulsive decision making) and non-planning (inability to maintain intentions and goals) (Barratt 1982). The inability to maintain inhibitory control over alcohol intake has been considered to be of fundamental importance for alcohol abuse (Fillmore and Weafer 2004; Finn et al. 2000; Jentsch and Taylor 1999; Lyvers 2000). Evidence indicates that the vulnerability to alcohol dependence may share a common genetic component with **antisocial personality disorder**, which may contribute to impulsive behavior and drug intake (Begleiter and Porjesz 1999; Bowirrat and Oscar-Berman 2005; Heinz et al. 2001; Pihl et al. 1993; Schuckit et al. 2004). Cloninger (1987) suggested that ***Novelty Seeking*** (NS) is a partially heritable and DA-related personality trait, which is associated with DA neurotransmission, and that high NS and low ***Harm Avoidance*** (HA) predisposes an individual to an early onset of alcoholism (Cloninger 1987a). Volkow et al. (2006) hypothesised that low DRD_2 availability is a partially heritable trait, which facilitates excessive alcohol and drug intake, while high DRD_2 levels appear to be protective against alcoholism. It was suggested that DRD_2 sensitivity is directly correlated with NS, however, this was not confirmed in controls and in patients suffering from alcohol dependence (Heinz et al. 1996).

Alcohol intoxication directly affects cognitive evaluation of the situation and impairs finding appropriate response strategies. It may result in disinhibited behaviors, poor self-control and inability to abstain drinking. Thus, excessive alcohol use interferes with executive and motivational functions that contribute to self-regulation and goal-directed behavior and can, subsequently, lead to further increase in alcohol intake. Consequently, impulsivity may facilitate excessive alcohol abuse both as a dispositional risk factor and as a consequence of excessive drinking (Oscar-Berman and Marinkovic 2007).

1.3 Effects of Chronic Alcohol Intake on Dopamine Neurotransmission

Why do some people use addictive drugs on an occasional non-addictive basis, while others suffer from an addictive pattern of use?

Genetic factors have been suggested to play an important role, accounting for 50% of the variance in people with clinically defined alcohol or drug addiction (Gardner 2005; Uhl et al. 1993). One hypothesis suggests that a ‘functional’ DA deficiency in the brain reward system derives from a genetically determined hypofunction of $\mathbf{DRD_2}$ gene [e.g., (Blum et al. 1996)]. This hypothesis was supported by human neuroimaging findings showing reduced DRD_2 levels in brain reward loci of drug addicts (Volkow et al. 2001, 1997, 1996), and from findings of low levels of DRD_2 in human brain reward loci, which predict rewarding versus non-rewarding subjective responses to psychostimulants (Volkow et al. 1999). However, while reduced DRD_2 sensitivity predicted relapse in alcohol-dependent

patients (Heinz et al. 1996), this DRD_2 down-regulation appeared to be a counter-adaptive down-regulation following **chronic alcohol intake**, which was not correlated with DRD_2 genotype (Heinz et al. 1995).

Additional studies performed to directly test the hypothesis that DRD_2 genotype is associated with alcohol dependence yielded mixed results, and it was suggested that genetic variation in DRD_2 expression does not predispose to alcoholism per se (Kienast and Heinz 2006).

1.3.1 Neuroadaptive Mechanisms

If alcohol intake induces DA release, chronic consumption should induce a compensatory **DA receptor down-regulation**, which can persist even after alcohol intake is stopped. Indeed, in detoxified alcohol-dependent patients, brain imaging studies with PET and endocrinological challenge studies revealed a reduction of sensitivity and availability of central DRD_2-receptors, which was correlated with lifetime alcohol intake and the subsequent relapse risk (Heinz et al. 1996).

Following detoxification, when the stimulating effects of alcohol on DA neurotransmission are interrupted, PET studies measuring F-DOPA showed that alcohol **craving** was specifically correlated with a low **DA synthesis capacity** and with reduced DRD_2 availability in the ventral striatum including the NAc (Heinz et al. 2005b, 2004b); (see Fig. 2). During detoxification and early abstinence, DA dysfunction is further exacerbated by reduced intra-synaptic DA release, as shown in rodent experiments where extracellular DA concentrations decreased rapidly during detoxification (Rossetti et al. 1992). A PET study confirmed that DA release following amphetamine administration was significantly reduced in detoxified alcoholics, indicating that presynaptic DA storage capacity is reduced during early abstinence (Martinez et al. 2005). Together, these studies indicate that after detoxification, overall DA neurotransmission in the ventral and central striatum of alcohol-dependent patients is reduced (rather than increased or sensitized, as might be expected from the theory of Robinson and Berridge (1993)). Nevertheless, this DA dysfunction appears to be associated with an increased neuronal response to drug-associated stimuli: in a multimodal imaging study combining PET and fMRI, ventral striatal DRD_2 down-regulation was not only correlated with the severity of alcohol craving but also with increased processing of alcohol-associated cues in the anterior cingulate and medial PFC (Heinz et al. 2004b). These brain areas are part of the attention network and an increased activation in these regions during the processing of alcohol cues has been associated with an increased relapse risk (Grüsser et al. 2004).

Moreover, DRD_2 down-regulation in the ventral striatum may interfere with the above described DA-dependent error detection signal (Schultz et al. 1997). Schultz et al. (1997) suggested that phasic alterations in DA release are not only required to learn new stimulus-reward associations but are also necessary to "unlearn" (extinguish) established associations. According to Schultz, a phasic dip of DA

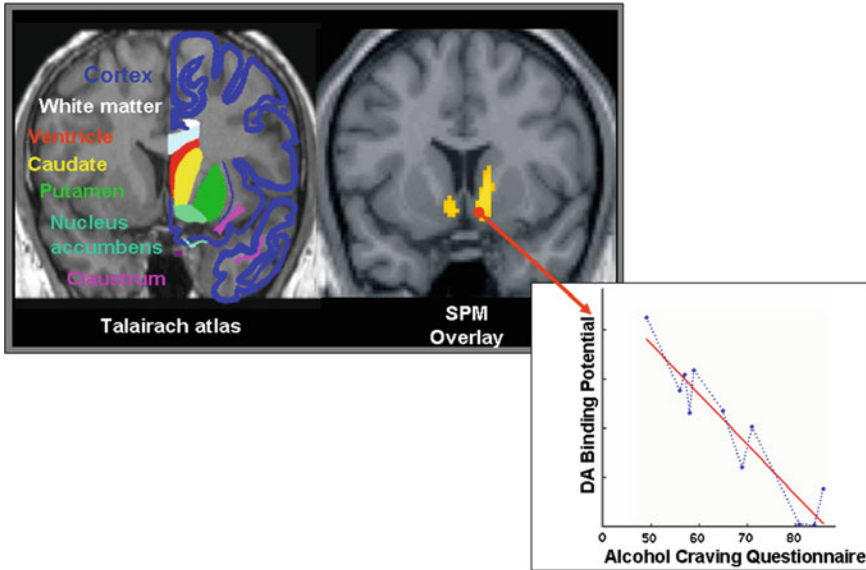

Fig. 2 Negative correlation between alcohol craving and DRD_2- availability in the bilateral nucleus accumbens/ventral striatum in a group of abstinent alcohol-dependent patients but not in healthy control subjects (modified according to Heinz et al. (2004b). The image at the upper right is a coronal view of the results of a PET correlation analysis of DRD_2 availability and alcohol craving measured with the alcohol craving questionnaire. The image at the upper left indicates that these areas correspond well to the ventral striatum/nucleus accumbens. The scatterplot at the bottom shows the correlation between DRD_2 availability (binding potential) in the right ventral striatum/nucleus accumbens and acute alcohol craving. Abbreviations: DA = dopamine; DRD_2 = dopamine D_2 receptor; PET = positron emission tomography; SPM = statistical parametric mapping

release occurs whenever a conditioned stimulus is not followed by the anticipated reward. Under this condition, the down-regulation of DA synthesis and storage and DRD_2 availability in the ventral striatum of detoxified alcoholics can interfere with this DA-dependent signalling of an error in reward expectation. Therefore, it may be difficult for alcoholics to divert their attention away from conditioned cues, which no longer signal subsequent delivery of any alcohol reward. The cues themselves may still elicit an orienting response even in the absence of DA firing due to cue-induced glutamate-dependent long-term potentiation (LTP) within a ventral hippocampus-ventral striatal pathway, which has been associated with perseverative behavior (Goto and Grace 2005). Thus, DA dysfunction following detoxification may specifically interfere with a phasic DA-dependent error signal dysfunction, which otherwise would indicate that alcohol-associated cues are no longer followed by reward. This may explain why patients continue to consume alcohol even though they no longer gain any rewarding experiences.

1.3.2 Morphological Alterations

Although functional and structural brain impairment is partially reversible after several weeks of abstinence (Crews et al. 2005; Hansson et al. 2010; Nixon 2006; Rosenbloom et al. 2003), the type and degree of damage varies across individuals (Oscar-Berman and Marinkovic 2007).

The most prominent damage in the frontal lobes is **cerebral atrophy**. Other morphological effects are **ventricular enlargement** and widening of the cerebral sulci of alcohol-dependent patients in relation to increasing age (Pfefferbaum et al. 1996; Sullivan 2000).

The majority of the evidence from neuropathological and neuroimaging investigations supports an increased vulnerability **model of "premature aging"** due to alcohol's neurotoxic effects (Oscar-Berman and Marinkovic 2003): when comparing older to younger alcohol-dependent patients, certain brain structures show greater than expected reduction in size (or blood flow), e.g., in the cerebral cortex (Di Sclafani et al. 1995; Harris et al. 1999; Pfefferbaum et al. 1997), in the hippocampus (Laakso et al. 2000; Sullivan et al. 1995), in the corpus callosum (Pfefferbaum et al. 2006, 1996; Schulte et al. 2005) and in the cerebellum (Harris et al. 1999; Sullivan 2000). At the microstructural level, **diffusion tensor imaging (DTI)** measures of neuronal fibers in the corpus callosum have provided evidence for a detrimental interaction between a persons recent history of alcohol dependence and their age (Pfefferbaum et al. 2006).

Such (micro) structural alterations could provide an alternative explanation for DRD_2 down-regulation: would D_2 receptors simply be reduced because of striatal atrophy, which leads to partial volume effects and thus decreases the signal from radioligand binding to DRD_2? This is certainly a possibility, but to date this type of striatal atrophy has not been described; also, measurements of mu-opiate receptors in the same brain area (ventral striatum) were increased rather than decreased in detoxified alcohol-dependent patients (Heinz et al. 2005a), suggesting that down-regulation of DRD_2 and up-regulation of mu-opiate receptors in the ventral striatum reflect specific neuroadaptive processes rather than simply resulting from striatal atrophy.

1.3.3 Changes in Cue-Induced Neuronal Activation

Alterations in incentive salience attribution to alcohol-associated stimuli can be assessed with **cue-reactivity paradigms** (Drummond 2000). Animal experiments revealed that besides the drug itself, also alcohol and drug-associated stimuli activate the DA reward circuitry including the ventral striatum (Dayas et al. 2007; Di Chiara 2002; Shalev et al. 2000). Brain-imaging studies have assessed the neuronal network responding to drugs of abuse, and its association with the prospective **relapse risk** (Braus et al. 2001; Drummond 2000; George et al. 2001; Grüsser et al. 2004). In the context of these cue-reactivity paradigms, it is practicable to examine conditioned reaction on conceptually

Table 1 Core regions activated by drug-associated stimuli during fMRI cue-reactivity paradigms

Brain structure	Function	Study
Anterior cingulate cortex (ACC) Adjacent medial prefrontal cortex (MPFC)	Attentional and memory processes Encoding of motivational value of stimuli	Grüsser et al. 2004 Heinz et al. 2004b Myrick et al. 2004
Orbitofrontal cortex (OFC)	Evaluation of reward and emotional value of stimuli	Myrick et al. 2004 Wrase et al. 2002
Dorsolateral prefrontal cortex (DLPFC)	Executive behavioral control Behavior control, e.g. when resisting craving Control of behavioral adaptations during learning processes	George et al. 2001 Park et al. 2010
Basolateral amygdala	Specification of emotional salience of stimuli Initiation of approach and avoidance behavior	Schneider et al. 2001
Ventral striatum (incl. NAc)	Motivational aspects of salient stimuli and association with motor responses	Braus et al. 2001 Wrase et al. 2002 Wrase et al. 2007
Dorsal striatum	Consolidation of stimulus-reaction patterns Habit formation	Grüsser et al. 2004 Modell and Mountz 1995

Abbreviation: fMRI = functional magnetic resonance imaging

different levels (Carter and Tiffany 1999): (1) a subjective level where anxiety, joy or craving can be evoked, (2) a physiological level measuring heart rate, skin conductance or functional brain activation and (3) a behavioral level, where the amount of alcohol intake or the latency until relapse can be observed (Wrase et al. 2006). In drug-dependent patients, it has been observed that drug-associated cues often elicit a physiological response similar to appetitive stimuli, although this does not automatically reflect conscious feelings of attraction or pleasure, and Lubman et al. (2009) showed that heroin users displayed reduced responsiveness to natural reinforcers across a broad range of psychophysiological measures.

Cue-induced functional brain activation can be indirectly assessed by measuring changes in cerebral blood flow with PET, by single photon emission computed tomography (SPECT) or by measuring the blood oxygen level dependent (BOLD) response with fMRI. Although these studies showed substantial variance in response toward the presentation of drug-associated stimuli, there are core regions which were activated in most studies (Weiss 2005); (for core regions see Table 1 and Fig. 3).

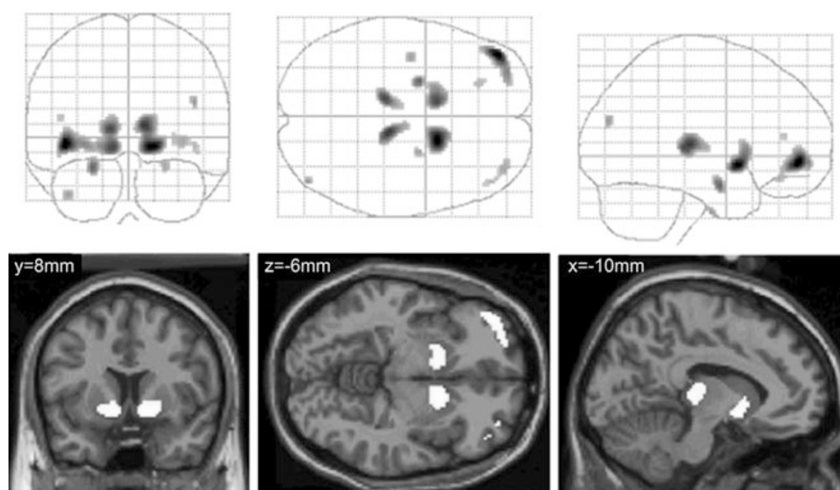

Fig. 3 Activation of the brain reward system assessed with fMRI during a cue–reactivity paradigm assessing the processing of alcohol-associated pictures in alcohol-dependent patients. Upper panel displays BOLD response in the bilateral ventral striatum, thalamus and prefrontal cortex for the contrast alcohol-related pictures versus neutral IAPS pictures in SPM glass brain. In the lower panel activation pattern for the same contrast superimposed upon a coronal, axial and sagittal plane averaged T1 structural image. Abbreviations: BOLD = blood oxygen level dependent response; fMRI = functional magnetic resonance imaging; IAPS = International Affective Picture System; SPM = statistical parametric mapping; T1 = T1-weighted magnetic resonance sequence

Findings concerning the association between cue-induced activity in these brain areas and subjective craving for EtOH are not consistent. In some studies, severity of craving was associated with functional brain activation in the ventral striatum, OFC and ACC (Myrick et al. 2004), dorsal striatum (Modell and Mountz 1995) or in the subcallosal gyrus (Tapert et al. 2004); other studies observed no significant correlation between alcohol craving and brain activation (Grüsser et al. 2004; Heinz et al. 2004b). These inconsistencies could be due to the diverse nature of the stimuli used in the studies: alcohol-related words (Tapert et al. 2004), alcohol-related pictures, either with (Myrick et al. 2004) or without (Grüsser et al. 2004) a sip of alcohol ("priming dose"). Moreover, patient recruitment state was not similar: in some studies, patients did not undergo detoxification and were able to consume larger amounts of alcohol, at least to a later time point (Myrick et al. 2004), while other patients were detoxified and participated in an inpatient treatment program, where relapse could cause termination of treatment (Braus et al. 2001; Grüsser et al. 2004; Heinz et al. 2004b, 2007; Wrase et al. 2002, 2007).

While a multitude of studies assessed brain activation during the presentation of alcohol-associated stimuli, only very few studies investigated to what extent brain activation elicited by alcohol or affective cues predicts an increased subsequent

relapse risk. In a pilot study by Braus et al. (2001), alcohol cues elicited increased activation of visual association centers and the ventral striatum in detoxified alcoholics compared to healthy subjects. Patients with a history of multiple relapses displayed stronger cue-induced activation of the ventral striatum than patients who had abstained from alcohol for longer periods of time. Grüsser et al. (2004) replicated these findings in a prospective study: subsequently relapsing patients showed an increased BOLD response elicited by alcohol-associated stimuli in the ACC and adjacent medial PFC and in the central (dorsal) striatum. These observations are in line with animal experiments, in which cue-induced relapse after cocaine consumption was prevented by blockade of DA and glutamatergic AMPA receptors in the dorsal rather than the ventral striatum (Vanderschuren et al. 2005). It has been suggested that the dorsal striatum is important for habit learning, i.e., for the learning of automated responses, and may thus contribute to the compulsive character of dependent behavior. In drug-addicted subjects, cue-induced craving preferentially elicit DA release in dorsal striatal structures (Volkow et al. 2006; Wong et al. 2006), reflecting the transition from ventral striatal, reward-driven behavior to dorsal striatal, stimulus–response habit formation (Berke and Hyman 2000). Robbins and Everitt (2002) suggested that although the initial reinforcing effects of drugs of abuse may activate the ventral striatum, in the course of drug consumption, the transition to habitual drug-seeking behaviors is reflected in a predominant role of the dorsal striatum in cue-responses. According to clinical experience, many patients describe their relapse in terms of such automated actions and do not remember experiencing any typical craving before the relapse occurred (Tiffany 1990).

If there is indeed DA dysfunction in detoxified alcohol-dependent individuals, which interferes with phasic changes in DA neurotransmission and reflects an error of reward prediction, patients should have difficulties to attribute salience to newly learned stimuli. This hypothesis was experimentally confirmed: while in detoxified alcohol-dependent patients the ventral and central striatum displayed increased neuronal activation during the presentation of alcohol-related stimuli (Braus et al. 2001; George et al. 2001; Kareken et al. 2004; Modell and Mountz 1995; Myrick et al. 2004; Wrase et al. 2007), brain activation in the striatum was reduced when the same patients were confronted with newly-learned cues, which indicated possible monetary reward (Wrase et al. 2007). Moreover, diminished activation of the ventral striatum was associated with the severity of alcohol craving. Decreased brain activation to newly learned, reward-indicating stimuli may thus interfere with the patients' motivation to experience new, potentially rewarding situations. Indeed, a reduced learning rate in alcohol-dependent patients correlated with a dysfunctional connectivity between ventral striatal error signalling and dorsolateral prefrontal cortical activation (Park et al. 2010). These findings are in accordance with the hypothesis that alcohol and other drugs of abuse "hijack" a dysfunctional reward system, which tends to respond too strongly to drug-associated cues while failing to process adequately natural reinforcers (Grace et al. 2007; Volkow et al. 2004; Wrase et al. 2007).

1.3.4 Long-Term Changes on Cognition/Emotion/Personality

Reduced activity of central DA transmission may be an underlying cause of negative mood states such as anhedonia, depression and dysphoria in alcohol-dependent patients (Heinz et al. 1994; Rossetti et al. 1992). These negative states can increase the risk of relapse in alcohol-dependent patients (Aneshensel and Huba 1983; Glenn and Parsons 1991). Contrary to this hypothesis, a study using **Cloningers Tridimensional Personality Questionnaire (TPQ)** (Cloninger 1987b) and neuroendocrinological challenge tests to assess DRD_2 sensitivity showed that hyposensitivity of central DA receptors was not associated with anhedonia, depression or anxiety. Instead, relapsing patients even showed a trend toward lower anxiety and depression scores compared to abstinent patients. The same study indicated that *harm avoidance* (HA) is not a stable personality trait, but in fact decreased significantly in all patients during observation. Although Cloninger (Cloninger 1987a, b) assumed that *novelty seeking* (NS) is influenced by central DA transmission, and although reduced DA sensitivity predicted relapse in detoxified alcohol-dependent patients, NS was not correlated with the sensitivity of central DA receptors. Also, there was no significant difference between NS scores of subsequently abstinent and relapsing patients before or after detoxification. Instead, reduced sensitivity of central DA receptors in relapsing patients seemed to be a consequence of long-term alcohol consumption and mostly disappeared within the first eight days of abstinence. Therefore, DA sensitivity seems to have an effect of alcohol-consuming behavior, which modulates motivational states such as craving for alcohol (Heinz et al. 2005b) rather than negative mood (Heinz et al. 1996). Depression and anxiety, on the other hand, were correlated with 5-HT dysfunction in alcoholism (Heinz et al. 1998).

In the domain of **emotion perception**, alcohol-dependent patients experience deficits in the processing of emotional facial expressions (Frigerio et al. 2002; Kornreich et al. 2002; Philippot et al. 1999; Uekermann and Daum 2008). An investigation of alcohol-dependent patients by Salloum et al. (2007) showed that patients displayed reduced functional activation while evaluating emotional facial expressions.

Two human studies simultaneously investigated brain activation elicited by both drug-associated cues and non-drug reinforcers such as sexual graphics or monetary reward in drug-addicted patients. One study revealed reduced brain activation elicited by the sexual graphics in cocaine-dependent patients, while brain activation elicited by drug-associated cues was increased (Garavan et al. 2000). Comparing detoxified alcohol-dependent patients with healthy control subjects, another study observed that alcohol-dependent subjects displayed increased activation of the ventral striatum during the presentation of affectively positive stimuli (Heinz et al. 2007). This ventral striatal activation appeared to have protective properties because it was inversely correlated with the number of subsequent drinking days and the amount of alcohol intake in the 6 month follow-up phase (Heinz et al. 2007). Therefore, it may be worth exploring protective effects of positive mood states on the relapse risk and their potential neurochemical underpinnings. Since, the hedonic

feeling of pleasure is associated with opioidergic rather than DA neurotransmission (Robinson and Berridge 1993); further studies may have to continue mu-opiate receptor and functional magnetic resonance imaging to assess opiate receptor effects on the processing of pleasant stimuli.

2 Summary

Altogether, animal experiments and human studies suggest that (1) DA function is prominent both when acquiring excessive alcohol intake and during chronic alcohol consumption. (2) Alcohol-induced DA release contributes to alcohol craving, while hedonic pleasure is mediated by other neurotransmitter systems, e.g., opioidergic neurotransmission. (3) Dopamine D_2 receptor down-regulation and low DA synthesis rates are at least partly neuroadaptive, compensatory mechanisms following chronic alcohol intake and correlate with reduced neuronal activation during reward expectancy, which is coupled with motivational and learning deficits. (4) DA dysfunction persists after detoxification for a limited amount of time (days to weeks) and can interfere with salience attribution to non-drug stimuli, while neuronal responses to alcohol cues remain elevated and predict the subsequent relapse risk. (5) An up-regulation of mu-opiate receptors in the ventral striatum contributes to chronic alcohol intake and craving. Dopamine–glutamate and dopamine–endorphin interactions remain to be further explored to optimize treatment strategies in alcoholism.

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Stimulant and Sedative Effects of Alcohol

Reuben A. Hendler, Vijay A. Ramchandani, Jodi Gilman and Daniel W. Hommer

Abstract Alcohol produces both stimulant and sedating effects in humans. These two seemingly opposite effects are central to the understanding of much of the literature on alcohol use and misuse. In this chapter we review studies that describe and attempt to measure various aspects of alcohol's subjective, autonomic, motor, cognitive and behavioral effects from the perspective of stimulation and sedation. Although subjective sedative and stimulatory effects can be measured, it is not entirely clear if all motor, cognitive and behavioral effects can be unambiguously assigned to either one or the other category. Increased heart rate and aggression seem strongly associated with stimulation, but motor slowing and cognitive impairment can also show a similar time course to stimulation, making their relation to sedation problematic. There is good agreement that alcohol's ability to induce striatal dopamine release is the mechanism underlying alcohol's stimulatory effects; however, the change in brain function underlying sedation is less well understood. In general, stimulatory effects are thought to be more rewarding than sedative effects, but this may not be true for anxiolytic effects which seem more closely related to sedation than stimulation. The two major theories of how response to alcohol predicts risk for alcoholism both postulate that individuals at high risk for alcohol use disorders have a reduced sedative response to alcohol compared to individuals not at high risk. In addition one theory proposes that alcoholism risk is also associated with a larger stimulatory response to alcohol.

Keywords Alcoholism · Sedation · Stimulation

R. A. Hendler · V. A. Ramchandani · J. Gilman · D. W. Hommer (✉)
Laboratory of Clinical and Translational Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA
e-mail: dan.hommer@mail.nih.gov

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Contents

1 The Nature of Stimulant and Sedative Effects 490
1.1 Effects on Subjective Experience 490
1.2 Effects on Autonomic Motor Activity 493
1.3 Effects on Motor and Cognitive Performance 494
1.4 Effects on Behavior 495
2 Specific Factors in Stimulant and Sedative Effects 496
3 Neurobiological Mediation of Alcohol's Effects 497
3.1 Stimulation 497
3.2 Sedation 499
4 Systems-Level Theories of Alcohol's Biphasic Effects 499
5 Clinical Implications: Risk Factors for Alcoholism and Alcohol Use Disorders 501
6 Directions for Future Research 502
References 503

Everyone knows that if you drink enough alcohol you get high and you get sleepy. The behavior resulting from the combination of these two states can be quite amusing; people have been laughing about alcohol's effects at least since Shakespeare's time. In *Macbeth* (Act 2, Scene 3), Shakespeare has a drunken porter tell an elaborate (and somewhat annoying) drunk joke at a dramatic high point in the plot. Scholars argue about why Shakespeare inserted the joke where he did, but there is no arguing with the porter's description of the effects of alcohol. The porter first mentions two of alcohol's effects not mediated by the central nervous system, increased urine production and flushing, but he's really interested in alcohol's ability to provoke sleep and lechery (not necessarily in that order). The joke is that alcohol provokes sexual desire but then takes away the ability to perform and puts you and your lechery to sleep. How does this happen? What are the brain mechanisms that allow alcohol to act as both a stimulant and a sedative?

In this chapter we will review some of the literature on the dualistic effects of alcohol. We will begin by discussing exactly what alcohol's effects are, focusing particularly on how alcohol induces stimulation and sedation. Scientists have assessed alcohol's effects on subjective experience, autonomic motor activity, motor and cognitive performance, and behavior. We will discuss the methods scientists have employed and the conclusions they have reached in studying each type of effect.

1 The Nature of Stimulant and Sedative Effects

1.1 Effects on Subjective Experience

Though subjective experiences are difficult to assess and quantify, alcohol researchers do their best using self-report measures. Research on alcohol-induced subjective effects generally involves having subjects consume alcohol and then

rate how they feel using a paper-and-pencil questionnaire. Several such questionnaires exist, each with its own strengths and weaknesses. The following are most commonly used.

The Biphasic Alcohol Effects Scales (BAES) was specifically designed to measure the stimulant and sedative effects of alcohol (Martin et al. 1993). The BAES assesses subjects' experience of seven subjective states associated with stimulation (elated, energized, excited, stimulated, talkative, up, and vigorous) and seven states associated with sedation (difficulty concentrating, down, heavy head, inactive, sedated, slow thoughts, and sluggish). Subjects rate their experience of each state from "not at all" to "extremely" on a scale from 0 to 10. Their ratings of stimulation and sedation are each summed into numerical scores which can be compared with a baseline score and analyzed mathematically. Studies have confirmed the reliability and validity of the BAES as a measure of alcohol-induced stimulation and sedation (Martin et al. 1993; Earleywine and Erblich 1996; Rueger et al. 2009).

The Profile of Mood States (POMS) measures natural mood states (e.g. "cheerful" and "grouchy"), on a five point scale from "not at all" to "extremely" (Speilber 1972). Several studies have shown that the POMS is sensitive to drug or alcohol-induced changes in mood (Johanson and Uhlenhuth 1980; Johanson and de Wit 1989; Nagoshi et al. 1991). Though the POMS measures some mood states probably irrelevant to stimulation and sedation (e.g. "lonely" and "sympathetic"), self-report of "elation" and "vigor" intuitively reflects stimulation and is associated with physiological stimulation (Conrod et al. 2001), preference for alcohol over placebo in a laboratory setting, and increased drinking behavior outside the lab (de Wit et al. 1987).

The Addiction Research Center Inventory (ARCI) measures the effects of specific classes of drugs (Martin et al. 1971), and has been shown to do so sensitively and reliably (Fischman and Foltin 1991). The ARCI consists of several dozen true/false statements, categorized by class of drugs. Subjects' answers in each category are summed to a scale score. The stimulant effects of alcohol can be measured using the Amphetamine scale, alcohol-induced euphoria can be measured using the Morphine-Benzedrine Group scale, and sedative effects can be measured using the Pentobarbital-Chlorpromazine-Alcohol Group scale (King et al. 2002).

The Drug Effects Questionnaire (DEQ) measures general drug effects and drug liking, though it does not measure stimulation and sedation directly. Subjects answer questions like "Do you feel any drug effects?" and "Would you want more of what you consumed, right now?" Rather than using a numerical scale, subjects indicate their response by drawing a mark on a 100 mm line, each end of which represents an extreme answer. The position of the mark is converted to a scaled score (King et al. 2002).

The Subjective High Assessment Scale (SHAS) was designed to measure subjective experience of drug or alcohol-induced intoxication (Judd et al. 1977a, b). The original version required subjects to answer 38 questions about their subjective state on a six point scale. Schuckit et al. have used a revised 13-item

version in studies to demonstrate differences in subjective effects of alcohol between individuals with family history of alcoholism and controls (Schuckit et al. 1996; Eng et al. 2005) as well as genetic influences on subjective responses to alcohol (Schuckit et al. 2005; Ray and Hutchison 2004). The SHAS measures subjective intoxication but does not differentiate between stimulation and sedation; some researchers believe that intoxication measured by the SHAS reflects sedation more than stimulation (Conrod et al. 2001).

Researchers compare subjects' experience of alcohol-induced stimulation and sedation by administering questionnaires at different points along the BAC curve. Using these self-report measures, researchers have found that people experience alcohol-induced subjective stimulation and sedation in a reliable pattern, although with substantial inter-individual variability in the magnitude and duration of the experience. In general, subjects experience greater stimulation than sedation during the ascending limb of the BAC curve (when BAC is rising), but when BAC peaks and begins to decline, sedative effects tend to overwhelm their initial stimulation (Pohorecky 1977; Babor et al. 1983; Martin et al. 1993; Earleywine and Erblich 1996).

Though stimulation and sedation seem like opposite states, they may actually occur simultaneously after alcohol consumption. Self-report measures like the BAES allow researchers to analyze the time-course of stimulation and sedation separately. Most subjects predominantly experience stimulant effects at low blood alcohol concentrations (BACs) soon after consuming alcohol. Subjects rapidly become increasingly stimulated until their BAC reaches its peak. As BACs decline, stimulation quickly wanes. In contrast, sedation takes effect slowly and gradually and predominates at high BACs. Sedation peaks and plateaus after peak BAC and declines gradually. The time course of stimulation and sedation are illustrated in Fig. 1.

Individuals differ, however, in when, how much, and under what conditions they feel stimulant and sedative effects after consuming alcohol (Holdstock and de Wit 1998). Individual response to alcohol can also change over time; studies have shown that humans and experimental animals can become more or less sensitive to stimulant drugs like cocaine and amphetamine over multiple periods of consumption (Robinson and Berridge 2000; Strakowski et al. 1996; Lett 1989; Nestler and Malenka 2004), and the same may occur with alcohol (Newlin and Thomson 1991, 1999).

Stimulant effects are generally experienced as positive and are believed to motivate drinking behavior (Corbin et al. 2008). Some sedative effects, like reduced anxiety, are also pleasant, but others, like motor impairment, are widely considered unpleasant (Morean and Corbin 2010). In general, stimulant effects are considered more positive than sedative effects. Individuals who experience mostly stimulant effects tend to experience more alcohol-induced euphoria, like alcohol more, and prefer alcohol to placebo more than individuals who experience mostly sedative effects (de Wit et al. 1987).

To complicate matters, however, sedative effects like reduced anxiety can also motivate drinking behavior. Many people initially drink primarily to experience

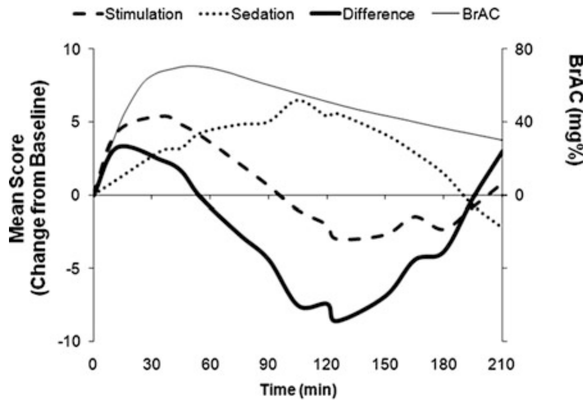

Fig. 1 Time course of Stimulation and Sedation Scores, as measured by the Biphasic Alcohol Effects Scale, following oral administration of a dose of 1 g/l total body water to 44 healthy social drinkers. Mean change from baseline scores are plotted on the primary *y-axis*. Mean breath alcohol concentration (BrAC, *thin solid line*) is plotted on secondary *y-axis*. Stimulation scale scores (*dashed line*) peak early (around 45 min) and decline to and slightly below baseline values by 90 min following the oral alcohol dose. Sedation scale scores (*dotted line*) show a slower change to a later peak (around 90 min) with a return to baseline by 180 min. The Difference (*thick solid line*) is the difference between stimulation and sedation scores and characterizes the biphasic effects of alcohol. (Ramchandani unpublished)

stimulation and accompanying positive affect but become dependent on alcohol when they switch to drinking primarily to experience reduced anxiety associated with sedation (Cooper et al. 1995; Schroder and Perrine 2007).

1.2 Effects on Autonomic Motor Activity

Alcohol-induced increases in heart rate (HR) often accompany subjective stimulation after alcohol consumption, and researchers consider alcohol's effects on HR a measure of alcohol-induced stimulation. By measuring heart rate at baseline and then after alcohol consumption, researchers have found that, like subjective effects, effects on HR differ in magnitude at different points along the BAC curve (Conrod et al. 2001, 1997a, b). Rapid consumption of alcohol tends to increase heart rate more during the ascending limb of the BAC curve than during the descending limb. After more gradual consumption of alcohol, however, HR can remain elevated during the descending limb (Conrod et al. 1997a, b).

Researchers have found that alcohol-induced increases in HR correlate with other measures of stimulation. For example, increases in HR correlate with subjective experience of energy and confidence near peak BACs (Conrod et al. 2001) and, to a lesser extent, with subjective stimulation on the rest of the BAC curve (Brunelle et al. 2007). Conversely, alcohol-induced increases in HR seem to

correlate negatively with experience of alcohol-induced sedation (King et al. 2002; Ray et al. 2006). Not surprisingly, then, individuals particularly sensitive to effects on HR tend to drink more than others (Conrod et al. 1997a, b).

Not all studies replicate these results, however. Ray et al. (2006) found no correlation between alcohol-induced increases in HR and stimulation on the BAES, though they did find increases in HR positively correlated with self-reported "vigor" on the POMS (Ray et al. 2006). Brunelle et al. found no correlation between alcohol-induced increases in heart rate and sedation at any point along the BAC curve (Brunelle et al. 2007). Some studies have raised concerns about the test-retest reliability of alcohol-induced increases in heart rate (Nagoshi and Wilson 1989; Wilson and Nagoshi 1987), although Conrod et al. have attempted to answer these concerns (Conrod et al. 2001). These discordant results may reflect differences in samples (e.g. different gender compositions, different numbers of heavy drinkers) and in methodology (e.g. subjective measures used, alcohol dose, method of administration, and measurement time points), so more controlled studies should be conducted to clarify alcohol's affect on autonomic motor stimulation (Brunelle et al. 2007).

1.3 Effects on Motor and Cognitive Performance

Alcohol impairs motor and cognitive performance. Performance impairment occurs differently on the ascending and descending limbs of the BAC curve, but it's unclear whether this impairment reflects stimulation or sedation. One might expect reduced motor control and slowed reaction time to reflect sedation; however, motor impairment peaks while stimulant effects are greatest, during rising BACs, and motor performance actually recovers as sedative effects dominate, during declining BACs (LeBlanc et al. 1975; Vogel-Sprott and Fillmore 1993).

Alcohol also impairs a host of cognitive functions, including attention, impulse-control, memory, and information processing (Jones and Vega 1972; Peterson et al. 1990; Hiltunen 1997a, b; Pihl et al. 2003; Soderlund et al. 2005; Schweizer et al. 2006; Schweizer and Vogel-Sprott 2008). Alcohol particularly impairs *executive cognitive functioning*, a collection of higher order cognitive abilities like planning, organization, abstract reasoning, cognitive flexibility, and self and social monitoring (Foster et al. 1994; Stuss and Benson 1984; Giancola and Zeichner 1997). Researchers assess these effects by measuring subjects' errors and response time on cognitive tasks.

Cognitive processes are impaired to different extents during rising and falling BACs, but, like motor impairment, not always in the ways one might expect. Reduced cognitive function might seem like sedation, but response time on cognitive tasks can actually return to baseline even while BACs are still declining (Nicholson et al. 1992; Schweizer and Vogel-Sprott 2008). Also, impairment with respect to the BAC curve seems to differ between cognitive processes. These differences may arise from differences in how alcohol affects the right and left

brain hemispheres; Schweizer et al. tested subjects on an extensive battery of cognitive tasks and found right brain processes like visual memory (Carlesimo et al. 2001) impaired primarily during falling BACs and left brain processes like verbal memory (Goldstein et al. 1988) primarily impaired during rising BACs (Schweizer et al. 2006). Though alcohol-induced motor and cognitive impairment differs between the ascending and descending limbs of the BAC curve like stimulation and sedation, researchers do not fully understand how impairment relates to stimulation and sedation.

1.4 Effects on Behavior

Researchers have also assessed alcohol-induced stimulation and sedation by measuring alcohol's effects on behavior. Research has particularly focused on alcohol's tendency to induce *aggressive* behavior, in part because alcohol consumption has been linked to a host of violent activities (Babor et al. 1983; Jacob and Leonard 1988; Leonard and Senchak 1993; Kaufman-Kantor 1990; Lindqvist 1986; Frances et al. 1986). Researchers often measure aggression in the laboratory using the Taylor aggression paradigm, in which subjects administer electric shocks to a fictitious opponent as part of a competitive task (Taylor 1967). Studies have consistently found that individuals who consume alcohol administer shocks of higher intensity and duration than those who consume a placebo or non-alcoholic beverage (Bushman and Cooper 1990; Kelly and Cherek 1993; Taylor and Chermack 1993). Comparing alcohol's effects on the ascending and descending limbs of the BAC curve, however, demonstrates that alcohol increases aggression during the ascending limb but *not* during the descending limb (Giancola and Zeichner 1997).

Aggression likely reflects stimulation rather than sedation, which may explain why alcohol induces aggression primarily while stimulant effects dominate, during the ascending limb of the BAC curve. Indeed, alcohol-induced aggression correlates with subjective stimulation (Giancola et al. 1998) and increased physiological arousal (Dengerin 1971; Donnerstein 1980; Edguer and Janisse 1994) while BACs are rising. However, aggression may also reflect alcohol's *depressant* effect on cognitive abilities, since poor cognitive functioning correlates with aggressive behavior independently of alcohol administration (Moffitt 1993; Seguin et al. 1995; Giancola and Zeichner 1994; Giancola et al. 1996).

Researchers have also assessed alcohol's stimulant and sedative effects by studying sexual and risk-taking behaviors following alcohol consumption. Alcohol has been shown to increase sexual arousal on subjective and physiological measures (Hull and Bond 1986) and is associated with increased sexual behavior and risky sexual behavior (Cooper 2002). Increased sexual arousal likely reflects stimulation, since it instigates approach behavior; consistent with this, alcohol increases sexual risk-taking intentions most during rising BACs (Davis et al. 2009). Consuming alcohol has also been shown to increase risky behavior in

general (Lane et al. 2004; Burian et al. 2002), but this may reflect a reduction in anxiety and related sedation rather than stimulation.

2 Specific Factors in Stimulant and Sedative Effects

We have so far mostly discussed research which focuses on how alcohol's stimulant and sedative effects differ between the ascending and descending limbs of a typical BAC curve. The ascending and descending limbs, however, differ in a variety of ways themselves, so a confluence of more specific factors may account for differences in alcohol's effects between them. For example, BACs change rapidly during the ascending limb but change slowly during the descending limb, and the ascending limb always precedes the descending limb in time. Researchers hypothesize that time, BAC, and rate of change in BAC may together determine an individual's response to alcohol. Indeed, alcohol's effects on heart rate and impairment of cognitive and motor abilities vary in magnitude depending on the rate of alcohol consumption (Friedman et al. 1980; Conrod et al. 2001). In addition, effects of alcohol have been shown to vary depending on the dose of alcohol consumed; low doses tend to induce stimulation, whereas high doses tend to induce sedation (Holdstock and de Wit 1998; Hiltunen 1997a, b; Pohorecky 1977).

Studies using oral administration of alcohol cannot easily isolate these factors, since time and rate of change in BAC constantly fluctuate after drinking. Recently, however, an intravenous infusion paradigm has been developed to overcome this limitation. In this "alcohol clamp" paradigm, subjects receive intravenous alcohol infusions at rates determined for each individual using computer models that take into account subjects' sex, weight, and other factors to bring subjects to a target BAC quickly and maintain that BAC for a period of up to several hours (Ramchandani et al. 1999, 2006). Holding BAC and rate of change in BAC constant, researchers can administer tests of subjective experience, autonomic motor activity, behavior, and cognitive ability repeatedly to evaluate the influence of time on alcohol's acute effects. The clamp paradigm provides exquisite control over the time course of BAC exposure and minimizes the inter-individual variability in alcohol pharmacokinetics that can result in 3- to 4-fold variability in BAC-time curves following oral administration of alcohol. The same oral administration can affect a subject very differently during different drinking sessions, but intravenous alcohol infusion can mimic a BAC curve which typically follows oral administration with greater control and reliability (Ramchandani et al. 2009). The clamp paradigm can be administered reproducibly over multiple sessions to further allow researchers to investigate the effects of repeated exposures on alcohol effects and the development of tolerance.

Evaluation of changes in BAES stimulation and sedation scale scores during an alcohol clamp study indicate that stimulation peaks before sedation even when BAC is held constant (Morzorati et al. 2002), suggesting that time may be an important factor in alcohol's stimulant and sedative effects independently of BAC.

The precise time course of stimulation and sedation, including the development of acute tolerance to these effects during constant exposure to alcohol, remains to be elucidated. Further studies should be also conducted to determine which factors are responsible for differences between alcohol's effects on the ascending and descending limbs of the BAC curve.

3 Neurobiological Mediation of Alcohol's Effects

3.1 Stimulation

The neurobiological mechanisms which mediate alcohol's stimulant effects are well understood. Researchers attribute these effects to activation of the brain's "reward circuitry," which motivates behavior, particularly approach behavior. Rewarding behaviors of all sorts are mediated by the release of the neurotransmitter dopamine in the ventral striatum and nucleus accumbens; this effect has been demonstrated with primary rewards like water and fruit juice (Berns et al. 2001; O'Doherty et al. 2002; Pagnoni et al. 2002; McClure et al. 2003), secondary rewards like money and praise (Knutson and Cooper 2005), and drugs of abuse like cocaine (Breiter et al. 1997) and nicotine (Stein et al. 1998). The dopamine reward circuit has been implicated in motivation (Di Chiara et al. 1992; Nader et al. 1997), stimulation (Enggasser and de Wit 2001), euphoria (Drevets et al. 2001), and addiction (Wise 1996; Esch and Stefano 2004) (Koob and Volkow 2010), and drugs or brain lesions that block dopamine release in these areas decrease many drugs' rewarding effects (Di Chiara 2000; Enggasser and de Wit 2001).

Alcohol works by activating dopamine release in this reward circuit. Studies using microdialysis in rats (Yim et al. 1998) and positron emission tomography (PET) in humans (Wang et al. 2000; Boileau et al. 2003; Schreckenberger et al. 2004; Ramchandani et al. 2010), have shown that consuming alcohol increases dopamine release and glucose metabolism in the ventral striatum/nucleus accumbens. There have been a few functional MRI studies measuring alcohol-induced changes in brain activity in this circuit as well. Using fMRI, Gilman et al. (2008) recently demonstrated activation of the ventral striatum/nucleus accumbens following acute IV infusion of alcohol as shown in Fig. 2.

Researchers believe that one of the ways alcohol promotes dopamine release is by activating μ-opioid receptors, which prevents GABAergic inhibition of dopamine in the midbrain (Spanagel 2009). As a result, drugs like naltrexone that block μ-opioid receptors reduce dopamine release following alcohol consumption (Gonzales and Weiss 1998; Heilig and Egli 2006).

Activation of the dopamine "reward circuit" has been shown to correlate with subjective and autonomic effects of alcohol. Subjective ratings of intoxication on the DEQ and stimulation on the BAES, for example, correlate with striatal

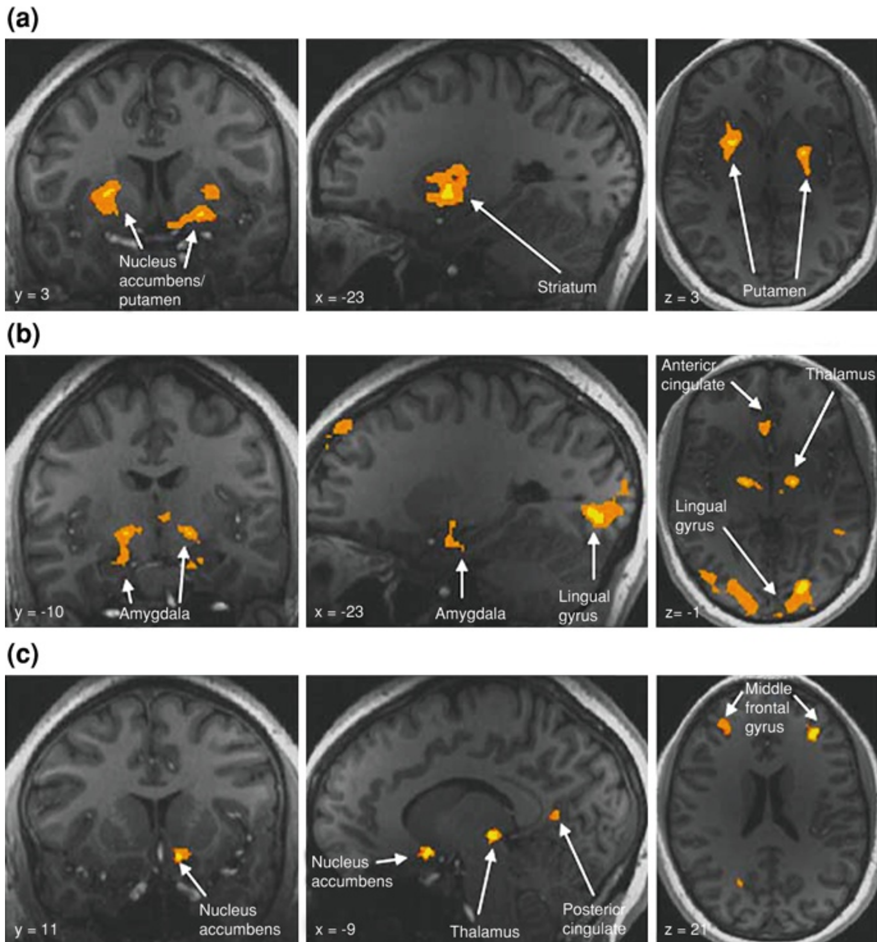

Fig. 2 Main effects of alcohol (**a**), fearful facial emotion (**b**), and the interaction between them (**c**) on regional brain activation. Anatomical maps of *t* statistics were spatially normalized by warping to Talairach space and combined into a group map. Radiological convention is used to display left and right. A statistical map of the main effects of alcohol and facial emotion was computed by performing a voxelwise ANOVA of the event-related β coefficients calculated from the general linear model. In this three-factor mixed-model ANOVA, alcohol (alcohol or placebo) and emotion (fearful or neutral) were fixed factors, and subject was a random factor. Alcohol effects were seen primarily in striatal areas, whereas emotion effects were seen in limbic and visual processing areas. The color map represents the *t* score: in *orange* regions, $p < 0.01$, and in *yellow* regions, $p < 0.001$. Reproduced from Gilman et al. (2008)

activation (Gilman et al. 2008; Yoder et al. 2005), and this circuitry seems to mediate alcohol-induced positive affect (Wise and Bozarth 1987; Di Chiara et al. 1992). PET studies have shown that this activation also correlates with alcohol-induced increases in HR (Boileau et al. 2003), which in turn reflect stimulation (Brunelle et al. 2007) and correlate with aggression (Assaad et al. 2006), desire for

alcohol in a laboratory setting, and heavy drinking behavior (Conrod et al. 1997a, b). In sum, alcohol-activated dopamine release in the ventral striatum seems to mediate alcohol-induced subjective experience of reward, behavioral stimulation, and increased heart rate. Termed "psychomotor stimulation," these effects are thought to motivate drinking behavior, implying that dopamine release in the ventral striatum/nucleus accumbens may underlie the desire to drink (Wise and Bozarth 1987; Conrod et al. 2001).

3.2 Sedation

Researchers know less about which brain mechanisms mediate alcohol's *sedative* effects. Sedation does not seem to arise simply from the dopamine reward circuit "turning off," since stimulation and sedation increase simultaneously after alcohol consumption (Conrod et al. 2001; King et al. 2002; Erblich et al. 2003; Holdstock and de Wit 1998). The pons, thalamus, and putamen are thought to play a role in anesthetic-induced sedation, which shares many similarities with sleep-induced sedation. However, it is unclear whether these same mechanisms mediate alcohol-induced sedation, and researchers in fact know relatively little even about the mechanisms that mediate sedation and anesthesia (Campagna et al. 2003; Mhuircheartaigh et al. 2010).

Some researchers hypothesize that alcohol-induced sedative effects reflect a general decrease in activity throughout the cerebral cortex. PET studies, for example, have shown that consuming moderate (Wang et al. 2000) or high (de Wit et al. 1990) doses of alcohol reduces cerebral glucose metabolism throughout the entire brain. Gradual, decentralized depression of brain activity could co-occur with more dramatic but short-term stimulant effects in the ventral striatum, resulting in the pattern of effects researchers observe.

Scientists have begun to localize brain inactivity related to salient sedative-like effects such as anxiolysis. Gilman et al. (2008), for example, used fMRI to show a blunting of the amygdala's ability to distinguish threatening stimuli from neutral stimuli, which may underlie the reduction in anxiety seen following alcohol use (see Fig. 2). Researchers hypothesize that alcohol may also reduce functioning in brain regions like the cerebellum, associated with motor coordination (Hanchar et al. 2005; Volkow et al. 1988; Boecker et al. 1996), and the frontal lobe, associated with higher order cognitive abilities (Peterson et al. 1990; Zorko et al. 2004).

4 Systems-Level Theories of Alcohol's Biphasic Effects

Researchers have also sought to explain the pattern of alcohol-induced effects from psychological and systems-level perspectives, with the following theories.

According to the theory of *acute tolerance*, people become less sensitive to the stimulant effects of alcohol over the course of a drinking session. In other words, the body compensates for alcohol-induced stimulation by increasing sedation to maintain a comfortable level of arousal. Acute tolerance can occur to behavior, as when subjects adapt to their intoxication during a drinking session and improve motor (LeBlanc et al. 1975; Vogel-Sprott and Fillmore 1993) and cognitive (Nicholson et al. 1992; Schweizer et al. 2006; Schweizer and Vogel-Sprott 2008) performance from the ascending limb of the BAC curve to the descending limb. Acute tolerance can also occur physiologically, as when subjects' HR increases during the ascending limb but returns to baseline during the descending limb (Conrod et al. 1997a, b). Researchers have some understanding of how heavy drinkers develop *chronic* tolerance to alcohol over time and repeated drinking sessions, but researchers are still investigating how one's physiology and behavior might similarly develop acute tolerance during a single drinking session. Recent work in mice has begun to identify mechanisms that underlie differences in acute functional tolerance (Hu et al. 2008).

According to the theory of *disinhibition*, alcohol directly depresses brain activity, but stimulation results when the brain reduces processing that inhibits behavior. Alcohol has been shown to interfere with inhibitory processes mediated by the amygdala, for example, which regulates fear and anxiety (Gilman et al. 2008; Moberg and Curtin 2009). The theory of disinhibition attributes increased social behavior, risk-taking, aggression, and positive affect after alcohol consumption to reduced anxiety. Alcohol's biphasic pattern of stimulation and sedation might reflect fast-acting sedation of inhibitory systems, followed by slower general sedation of the entire brain (Graham 1980; Giancola and Zeichner 1997).

According to the theory of *alcohol myopia*, alcohol-induced reduction in attentional resources explains many of alcohol's effects. This theory attributes alcohol-induced reduction in anxiety and the accompanying increase in social behavior, risk-taking, aggression, and euphoria to reduced self-consciousness (Hull 1981). In alcohol myopia, people only have sufficient attentional resources to focus on the most salient stimuli while ignoring subtle or peripheral information. Alcohol might decrease inhibition if the most salient cues instigate action and inhibitory clues tend to be more subtle (Steele and Josephs 1990; Taylor and Leonard 1983; Giancola and Zeichner 1997).

According to *alcohol expectancy theory*, alcohol induces drunken behavior mainly because drinkers *believe* that it will. Anyone who has observed someone stumbling around after only one drink might be initially persuaded. The theory suggests that drinkers might experience stimulation initially because they believe alcohol makes them high and experience sedation later when alcohol's physiological, sedative effects set in. Many studies indicate that expectancy influences alcohol's effects (MacAndrew 1969; Lang et al. 1975; Pihl et al. 1981; Pihl 1983; Hull and Bond 1986; Fillmore and Vogel-Sprott 1998), but meta-analyses show that some types of alcohol effects are likely unaffected by expectancy, including increased heart rate, psychomotor slowing and aggression (Bushman 1993; Bushman and Cooper 1990; Hull and Bond 1986; Steele and Southwick 1985).

5 Clinical Implications: Risk Factors for Alcoholism and Alcohol Use Disorders

Why does any of this matter? Behind only tobacco use and obesity, alcohol use and abuse is the third most common lifestyle-related cause of death in the United States (Mokdad et al. 2004). Researchers suspect that individual differences in susceptibility to alcohol's effects may partially explain why some people drink excessively and others don't. Understanding what about certain people makes them drink excessively could help clinicians identify individuals at risk for alcohol use disorders and create treatments for these disorders that reduce alcohol's reinforcing effects (de Wit et al. 1987). Towards this end, researchers have attempted to identify how individuals at elevated risk for alcoholism experience alcohol differently than others.

Research has focused on two populations at high statistical risk for alcoholism: children of alcoholics and heavy drinkers. Though most children of alcoholics do not become alcoholics themselves, they are significantly more likely than children of non-alcoholics to develop alcoholism (Cotton 1979). Though socio-cultural factors may contribute to this trend, researchers suspect that genetics also plays a role, and the same genes that predict increased risk for alcoholism may also mediate a distinctive response to alcohol (Newlin and Thomson 1990). Heavy drinking is a risk factor for the development of alcohol dependence and is inherently hazardous (Holdstock et al. 2000; King et al. 2002).

Researchers have proposed two main hypotheses about how alcohol affects individuals at risk for alcoholism differently than others. According to the *low level of response hypothesis* (LLR), advanced by Schuckit and colleagues, individuals at risk for alcoholism tend to be less sensitive to alcohol's effects than others (Schuckit and Smith 2000; Schuckit 1980, 1994). Schuckit found that children of alcoholics exhibit greater motor control after drinking and report less intoxication on the SHAS than controls. He also found that individuals who report less intoxication on the SHAS than controls after consuming a particular dose of alcohol are more likely than controls to become alcoholics (Schuckit 1994) (Schuckit and Smith 2000; Schuckit et al. 2004). The LLR hypothesis suggests that individuals with low sensitivity to alcohol may drink more than their peers to experience the same psychomotor effects. Drinking excessively, these individuals may develop chronic tolerance, and, needing to consume ever more alcohol to feel the same effects, they may ultimately develop alcohol use disorders.

Critics of the LLR hypothesis, however, point out two potential limitations in Schuckit's findings (Crabbe et al. 2010). First, the studies typically assess alcohol's effects using the SHAS, which allegedly measures sedation accurately but not alcohol-induced stimulation (Conrod et al. 2001). Second, the studies primarily measure subjects during the descending limb of the BAC curve, while sedative effects dominate (Schuckit 1984, 2009). Thus, the findings of Schuckit's studies appear to show primarily that individuals at risk for alcoholism are less sensitive to its sedative effects (Newlin and Renton 2010).

Many researchers favor a different theory. First proposed by Newlin and Thomson (1990), the *differentiator model* (DM) states that individuals at risk for alcoholism are less sensitive to alcohol-induced sedation than others but *more* sensitive to alcohol-induced stimulation. The DM suggests that, since people usually like stimulant effects and dislike sedative effects, individuals who experience much stimulation after alcohol consumption but little sedation will usually like alcohol and drink more than most people.

A variety of studies support the DM in whole or in part (Erblich et al. 2003; Holdstock et al. 2000; DeWit et al. 1989; Conrod et al. 1998; Morzorati et al. 2002). Stimulant effects correlate with activation of the dopamine reward circuit and psychomotor stimulation (Wise and Bozarth 1987; Di Chiara et al. 1992), as well as euphoria (Drevets et al. 2001), drug liking, "wanting more" of a drug (King et al. 2002), and behavioral preference for ethanol (de Wit et al. 1987; Enggasser and de Wit 2001). Studies also show that alcoholics and children of alcoholics are particularly sensitive to alcohol-induced increase in heart rate, a measure of psychomotor stimulation (Finn et al. 1990; Conrod et al. 1995, 2001; Peterson et al. 1996; Newlin and Thomson 1999). It should be noted, however, that people who drink primarily to reduce anxiety develop alcohol use disorders more frequently than those who drink to enhance positive mood, despite both theories' suggestion that at-risk drinkers are less sensitive than others to alcohol's sedative effects (Cooper et al. 1995; Schroder and Perrine 2007). More longitudinal studies should be conducted to clarify which responses to alcohol constitute risk factors for the development of alcohol use disorders. In addition, careful exploration of the similarities and differences between animal models of alcohol sensitivity and the human phenomena would be of considerable value (Crabbe et al. 2010).

6 Directions for Future Research

Researchers have measured stimulant and sedative effects on subjective experience, behavior, autonomic motor activity, motor and cognitive performance, and the brain. Time, dose, BAC, rate of change in BAC, and limb of the BAC curve have all been found to influence the magnitude and time-course of alcohol effects. Researchers have identified the neurobiological mechanisms that mediate alcohol's stimulant effects and explained them with several systems-level theories. To explore clinical solutions to alcohol use disorders, researchers have identified responses to alcohol correlated with elevated risk. Where do we go from here?

Relatively few studies have made use of the newest and most sophisticated tools available for studying alcohol: imaging (fMRI and PET) and intravenous infusion paradigms. fMRI may prove invaluable in localizing alcohol's sedative effects and learning more about the mechanisms by which alcohol activates dopamine release to induce stimulant effects. PET studies would help improve our understanding of the neurochemistry (neutrotransmitters and receptor systems)

underlying the effects of alcohol. Intravenous infusion methods provide experimental control over the alcohol exposure and exploiting this control with novel exposure paradigms may isolate the factors that influence alcohol-induced stimulation and sedation and help determine individual responses that increase risk for alcohol use disorders. By studying why people drink, researchers hope to learn how to control the effects of alcohol and reduce the prevalence of alcohol use disorders.

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Chronic Alcohol Consumption, Abstinence and Relapse: Brain Proton Magnetic Resonance Spectroscopy Studies in Animals and Humans

Dieter J. Meyerhoff, Timothy C. Durazzo and Gabriele Ende

Abstract This chapter summarizes the peer-reviewed literature of proton magnetic resonance spectroscopy (^{1}H MRS) studies on the effects of chronic and excessive alcohol consumption in both the animal and human brain. After a brief summary of the neuropathology of alcohol use disorders (AUD), we describe the primary brain metabolites measured by in vivo ^{1}H MRS. We then focus on published MRS studies of animal models of alcohol dependence and of treatment-seeking humans with AUD. We also summarize the scant MRS research on the much larger fraction of treatment-naïve individuals with AUD and the similarities and discrepancies relative to treatment-seekers. It is exceedingly apparent that premorbid and/or comorbid disorders/conditions, especially chronic smoking, among individuals with AUD contribute to the considerable variability in the pattern and magnitude of neurobiological and neurocognitive abnormalities in AUD. Therefore, we also review studies on the neurobiological consequences of the combined effects of chronic drinking and smoking in AUD. Finally, as AUD is characterized by a chronically relapsing/remitting course over lifetime and identification of those at greatest risk for relapse is important, we review ^{1}H MRS studies on brain spectroscopic measures that contribute to the prediction of relapse in AUD. We conclude with an

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D. J. Meyerhoff (✉) · T. C. Durazzo
Center for Imaging of Neurodegenerative Diseases,
San Francisco Veterans Affairs Medical Center,
San Francisco, CA, USA
e-mail: dieter.meyerhoff@ucsf.edu

D. J. Meyerhoff · T. C. Durazzo
Department of Radiology and Biomedical Imaging,
University of California San Francisco,
San Francisco, CA, USA

G. Ende
Department of Neuroimaging, Central Institute of Mental Health,
Mannheim, Germany

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overall assessment of the MRS research literature on brain alcohol effects, the role of animal and human studies in understanding the disease, and discuss the need of widely integrative MRS studies of cohorts that include individuals with comorbidies that are reflective of the general population with AUD.

Keywords Alcoholism · Alcohol use disorders · Magnetic resonance spectroscopy · Neuroimaging · Brain metabolites · Recovery · Relapse · Nicotine · Smoking

Abbreviations

AUD	Alcohol use disorders
CNS	Central nervous system.
Cho	Choline-containing compounds
Cr	Creatine and phosphocreatine
CSF	Cerebrospinal fluid
GABA	Gamma aminobutyric acid,
Glu	Glutamate
GM	Gray matter
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
mI	Myoinositol
MR	Magnetic resonance
MRI	Magnetic resonance imaging
WM	White matter
^{1}H MRS	Proton magnetic resonance spectroscopy.
^{1}H MRSI	Proton magnetic resonance spectroscopic imaging

Contents

1 Introduction 513
2 Neuropathology of AUD 514
3 Proton MRS Methods and Commonly Measured Brain Metabolites 515
4 The Neurobiological Consequences of AUD and Changes During Abstinence from Alcohol 519
5 Treatment-Seeking Versus Treatment Naïve Alcoholics and Common Comorbidities 527
6 The Neurobiological Correlates of Relapse in Alcohol Use Disorders (AUD) 529
7 Conclusions and Perspectives 530
References 532

1 Introduction

This chapter summarizes the peer-reviewed literature on proton magnetic resonance spectroscopy (MRS) studies on the effects of chronic and excessive alcohol consumption in both the animal and human brain. After a brief summary of the neuropathology associated with alcohol use disorders (i.e., alcohol abuse and dependence), we describe the primary metabolites measured by in vivo proton MRS. We then focus on published MRS studies of animal models of alcohol dependence and of humans with alcohol use disorders (AUD). The human studies focus on individuals with uncomplicated AUD, i.e., without a history of Wernike-Korsakoff Syndrome or encephalopathy secondary to chronic hepatic disease. The neuropathological, neuroimaging and neurocognitive corollaries of Wernike-Korsakoff Syndrome and alcohol-induced hepatic encephalopathy have been reviewed elsewhere (Behar et al. 1999; Harper et al. 2003; Hazell and Butterworth 1999; Oscar-Berman 2000; Sullivan 2000; Thomson and Marshall 2006).

It is estimated that only approximately 10% of individuals with AUD seek treatment at some point in their lives (Fein and Landman 2005; Moss et al. 2007). However, most of what is known about the effects of AUD on the human brain is derived from studies with individuals recruited from inpatient and outpatient treatment programs (Fein et al. 2002), only a small fraction of whom participate in research studies. Given the possible bias from deriving potentially clinically useful data from a very small minority of affected individuals, we also summarize the scant MRS research on the much larger fraction of treatment-naïve individuals with AUD and the similarities and discrepancies relative to treatment-seekers.

In this context, it is widely recognized from large United States-based epidemiological studies, such as the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), that a number of premorbid and/or comorbid disorders/conditions are associated with AUD (Hasin et al. 2007; Mansell et al. 2006; Mertens et al. 2003; Stinson et al. 2005). These together may promote considerable variability in the pattern and magnitude of neurobiological and neurocognitive abnormalities demonstrated in AUD. Since chronic cigarette smoking is the most common comorbidity (Daeppen et al. 2000; John et al. 2003; Room 2004), we also review studies on the neurobiological consequences of the combined effects of chronic drinking and smoking.

Finally, AUD is characterized by a chronically relapsing/remitting course over lifetime (Dawson et al. 2007; Maisto and Connors 2006; Zywiak et al. 2006). A substantial amount of research has investigated the psychological, psychiatric, sociodemographic and behavioral correlates of relapse following treatment; however, the neurobiological factors contributing to relapse in humans have only recently begun to be delineated, largely due to advances in in vivo neuroimaging techniques (Durazzo et al. 2010b). We briefly summarize the applicability of proton MRS to the prediction of relapse in AUD. We conclude with an overall assessment of the MRS research literature on brain alcohol effects, the role of animal and human studies in understanding the disease, and discuss the need of

MRS studies of cohorts that include individuals with comorbidities that are reflective of the general population of those with an AUD.

2 Neuropathology of AUD

Postmortem examinations of individuals with uncomplicated AUD indicate neuronal loss primarily in the dorsolateral frontal cortex, hypothalamus and the cerebellum, with the hippocampi showing glial rather than neuronal loss (Harding et al. 1997; Korbo 1999; Kril and Halliday 1999; Kril et al. 1996). Reduced glial cell density and size in the dorsolateral prefrontal cortex (Miguel-Hidalgo et al. 2002) and lower neuronal and glial cell density in the orbitofrontal cortex have also been reported (Miguel-Hidalgo et al. 2006). However, other investigators found no abnormalities in neocortical neuronal cell volumes, neuronal and glial cell numbers or lobar and global neocortical surface area, thickness and volume in postmortem studies of AUD (Fabricius et al. 2007; Jensen and Pakkenberg 1993). White matter (WM) loss in anterior brain regions has also been reported, which may involve disturbances in both myelin and axonal integrity (Harper 2009). According to a general model by Harper and Kril (1989), alcohol-related cortical brain damage either constitutes loss of dendritic arbor and shrinkage of neuronal cell body volume or neuronal death and Wallerian degeneration of myelinated axons (e.g., Schwab and Bartholdi 1996) and occurs primarily in the frontal lobe, particularly in the superior frontal cortex (Harper 2009). With abstinence from alcohol, dendritic arbor and neuronal cell body volume increases have been reported, as have changes in myelin structure and increases in tissue density, particularly in the neocortical and subcortical gray matter (e.g., Dlugos and Pentney 1997; Sullivan and Pfefferbaum 2005). This suggests that the brain does recover from alcohol-induced brain injury with extended sobriety.

The mechanisms of chronic alcohol-associated brain injury and neurocognitive dysfunction are hypothesized to involve glutamate and homocysteine-induced excitotoxicity, reduced levels of brain derived neurotrophic factors, increased oxidative stress and free radical levels, thiamine and other nutritional deficiencies, increased acetaldehyde and aldehydes levels, hepatic dysfunction and genetic vulnerability (for review see Durazzo and Meyerhoff 2007). Excitotoxicity has been suggested to be most prominent during withdrawal from alcohol (De Witte 2004; Harris et al. 2003; Prendergast et al. 2000). In human AUD, reports of associations between level of alcohol consumption and structural, metabolic and functional brain injury are inconsistent (Durazzo and Meyerhoff 2007).

More recently, however, rodent studies have suggested that mechanisms of alcohol-related brain injury involve oxidative stress secondary to proinflammatory enzymes that are operative during intoxication rather than withdrawal (Crews and Nixon 2009). In a rodent model, Crews et al. (2004) have shown that binge ethanol administration induces brain damage demonstrated by agyrophilic silver staining. Brain damage increases progressively after 2 days beginning in the olfactory bulb

followed by additional brain regions showing increasing damage with further binge exposure. The rodent binge model shares both neurodegenerative and cognitive deficits found in the human alcoholic brain (Crews and Nixon 2009). Prolonged alcohol dependence in rats has also been linked to long-term suppression of forebrain neurogenesis and loss of neuronal progenitor cells (Hansson et al. 2010). In studies of moderate alcohol consumption, alcohol-preferring rats were given the choice of drinking 10% alcohol in water or pure water for 7 weeks (He et al. 2009). During the subsequent abstinence period, hippocampal neurogenesis increased as did differentiation of oligodendrocyte progenitors in the cingulate and proliferation of undifferentiated cells in the substantia nigra. Such cellular alterations associated with alcohol dependence and abstinence may contribute to alcohol-induced cortical dysfunction and neurocognitive deficits as well as to their recovery during prolonged sobriety.

All of the potential mechanisms mentioned above may work independently or together in AUD to alter cerebral cellular structures or organelles, membrane phospholipids, myelin, DNA, gene expression, protein synthesis and cellular metabolism.

3 Proton MRS Methods and Commonly Measured Brain Metabolites

Magnetic resonance spectroscopy (MRS) enables the non-invasive and concurrent quantitation of several metabolites from most brain regions. Proton MRS (^{1}H MRS) enables the assessment of neurophysiological consequences of a disease/condition that may precede any associated gross morphological changes. Most studies have been performed with single-volume approaches that acquire MR spectra from one or a few volumes of interest within the brain, with typical volumes between 4 and 16 cm^3. The more technically and analytically demanding two- and three-dimensional MR spectroscopic imaging (MRSI, also called chemical shift imaging or CSI) approaches use a combination of phase encoding and spectroscopy to acquire simultaneously MR spectra from up to hundreds of volumes from throughout most of the brain, with typical volumes of 0.8–2 cm^3. The latter approach also allows reconstructing metabolite images, which display the distribution of metabolites throughout the imaged region. Most of the MRS- and MRSI-detectable brain proton metabolites addressed below are associated with neurocognition in normal aging, substance/alcohol use disorders, neurodegenerative diseases, psychiatric conditions and traumatic brain injury (Babikian et al. 2006; Durazzo and Meyerhoff 2007; Martinez-Bisbal et al. 2006; Ohrmann et al. 2008; Ross and Sachdev 2004; Schuff et al. 2006; Yildiz-Yesiloglu and Ankerst 2006a, b; Zahr et al. 2008). These relationships demonstrate the functional relevance and clinical significance of MRS measurements (Steen et al. 2005).

At a magnetic field strength of 1.5 Tesla (T), the following brain metabolites are most frequently measured: N-acetylaspartate (NAA), choline-containing compounds (Cho), creatine-containing compounds (Cr) and myo-inositol (mI; with

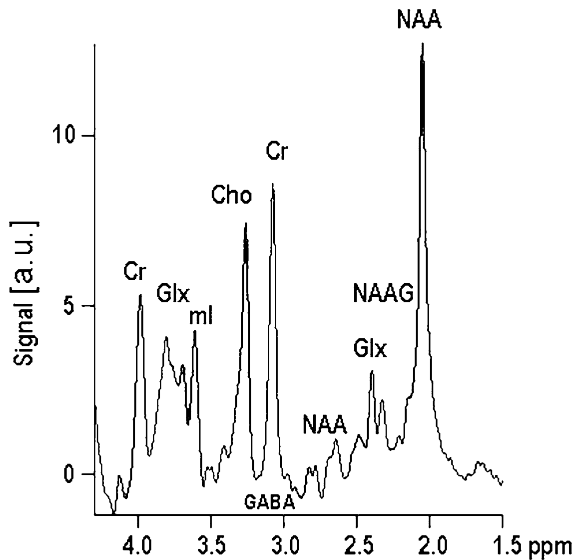

Fig. 1 Exemplary brain ^{1}H MR spectrum obtained at 3 T with 30 ms echo time. The signal integral (*here shown in arbitrary units, a.u.*) is proportional to the concentration of the metabolite that gives rise to the resonance. All major resonances are labeled. NAA = N-acetylaspartate, NAAG = N-acetylaspartyl-glutamate (*as a shoulder on the left side of the NAA resonance*), Glx = Glutamate + Glutamine (which can be separately observed at 4 T), GABA = gamma-aminobutyric acid (*as a shoulder on the right side of the Cr resonance*), Cr = Creatine + Phosphocreatine, Cho = choline-containing metabolites, mI = myo-Inositol

short echo time pulse sequences). At higher magnetic field strengths (e.g., >2T), the general metabolic pool of glutamine, glutamate (Glu) and gamma aminobutyric acid (GABA) can be measured. Figure 1 depicts an exemplary ^{1}H MR spectrum obtained at 3 T with the major metabolite peaks labeled. The peak areas of the individual signals are directly proportional to the concentrations of these metabolites at the location of the spectral measurement. The metabolite concentrations are often given as ratios (e.g., NAA/Cr) and increasingly as absolute or relative/semi quantitative concentrations; there are advantages and disadvantages associated with either quantitation approach. It is procedurally and computationally less demanding to calculate metabolite ratios, however, interpretation of group differences, for example, become unclear if both nominator and de-nominator are affected by the disease under consideration. Absolute quantitation of individual metabolite concentrations does not have this disadvantage, but requires careful and more labor intensive calibration of concentrations based on measured amounts of tissue and cerebral spinal fluid in the spectroscopy volumes, tissue water content, and potential receiver and transmitter gain differences between individuals. For further details see (Jansen et al. 2006).

NAA is an amino acid derivative that is found in high concentrations in axons and dendrites of neurons, particularly in pyramidal neurons, and it is virtually absent in mature glial cells (Benarroch 2008; Moffett et al. 1991; Simmons et al.

1991). The in vivo MRS peak for NAA is composed of overlapping signals from the prominent NAA and the less pronounced N-acetylaspartylglutamate (NAAG). NAA is synthesized in the neuronal mitochondria, from where it is exported to the cytosolic compartment. In the extracellular fluid it diffuses to oligodendrocytes, where it is rapidly hydrolyzed by amidohydrolase II (Baslow 2003). NAA acts as an organic osmolyte controlling cellular water distribution, which provides a critical source of acetate for myelin lipid synthesis in oligodendrocytes (myelinogenesis) and is involved in facilitating energy metabolism in neuronal mitochondria. NAA is also an immediate precursor for the enzyme-mediated biosynthesis of the neuronal dipeptide NAAG, which acts to regulate glutamate and dopamine release, most likely via activation of presynaptic mGluR2/3 receptors. Abnormalities in NAA synthesis, transport and/or breakdown (i.e., neuronal dysfunction) may contribute to an abnormal steady-state concentration that is measurable by ^{1}H MRS/MRSI. The MRS signal from NAA is often described as a marker of neuronal viability or integrity (Baslow and Guilfoyle; De Stefano et al. 1995; Hugg et al. 1996; Schuff et al. 2001; Sullivan 2000; Vion-Dury et al. 1994). However, nominal NAA levels do not seem to be mandatory for neuronal viability or function because differentiated cultured neurons were still viable in the absence of NAA and a progressive loss of NAA was detected in viable cultured organotypic brain slices (for review see Baslow 2003). Recent studies in various pathologies have shown that NAA reduction can be at least partially reversible, which suggests that NAA does not necessarily reflect the density or concentration of neurons per se but is rather sensitive to the plasticity of neuronal components and pathological processes affecting the metabolic functioning of neurons (Bertolino et al. 2003), particularly neuronal bioenergetics (Baslow and Guilfoyle 2007; Pan and Takahashi 2005). Additionally, postmortem studies showed that NAA reduction is correlated with the overall volume of neuronal soma size (Rajkowska et al. 1998; Selemon and Goldman-Rakic 1999).

NAA has been shown to be distributed homogeneously throughout the brain, at a concentration of approximately 10 mmol/L, whereas NAAG increases rostral to caudal (1.5–2.7 mmo/L) and exhibits higher concentrations in WM than in gray matter (GM) (Pouwels and Frahm 1998). Whether the NAA concentration is higher in GM or WM is a topic of continued debate and appears critically dependent on region and method. NAA tends to decrease globally throughout the brain with age (Maudsley et al. 2009) and has been shown to decrease as a function of age in the temporal lobe (Riederer et al. 2007) and the medial prefrontal brain (Ende et al. 2000).

The MR detectable Cho represents the trimethyl ammonium resonance of several choline-containing compounds. Most of the proton MRS resonance is contributed from phosphocholine and glycerophosphocholine, with free choline contributing less than 5% and the neurotransmitter acetylcholine even less (Boulanger et al. 2000). The choline-containing compounds are intermediates in phospholipid (membrane) synthesis and breakdown, and it has been suggested that decreased phosphocholine and increased glycerophosphocholine levels (together with other phosphoester alterations) reflect membrane breakdown (Pettegrew et al. 1987, 1990). Phosphatidylcholine, the major choline-containing metabolite of the

brain and the main component of myelin, cell membranes, and other brain lipids is invisible under normal MRS acquisition conditions, as it is restricted in its molecular mobility. However, in ^{1}H MRS, (non membrane bound) choline-containing compounds cannot be distinguished—all are detected within one Cho peak. The compounds exhibit a marked regional variability with the highest concentrations in the cerebellum and lowest levels and a strong rostral to caudal decreasing gradient in GM (Pouwels and Frahm 1998). The Cho concentration is between 1.5 and 2.5 mmol/L and has been shown to increase with age (Maudsley et al. 2009). A high Cho signal is thought to reflect increased cellular membrane turnover and density (Miller et al. 1996), myelin catabolism (Ross and Bluml 2001) and/or inflammation (Brenner et al. 1993).

In its bioactive form, myo-inositol (mI) is a carbohydrate that structurally resembles glucose. mI is a constituent of phosphatidylinositol, an important component of the phospholipid bilayer that constitutes all eukaryotic cell membranes. mI is synthesized predominantly by glia in the brain and is incapable of crossing the blood–brain barrier (Brand et al. 1993). The biological significance of mI has not yet been established with certainty. It has been suggested to be a glia-specific marker (Brand et al. 1993) and/or an osmolyte (Ross and Bluml 2001; Schweinsburg et al. 2000) and to be involved in second messenger system functioning (Fisher et al. 2002). mI elevations may reflect inflammation, astrocyte proliferation and/or an osmotic response to cell shrinkage (Rosen and Lenkinski 2007).

The MRS signal of Cr derives from creatine plus phosphocreatine. In normal brain metabolism, phosphocreatine supplies a phosphate group to adenosine diphosphate (ADP), resulting in the production of adenosine triphosphate (ATP) and the release of creatine. Thus, total creatine should be a reliable marker of brain metabolism, reflecting bioenergetics of neuronal and glial tissue (Ferguson et al. 2002). Creatine, phosphocreatine, and their main precursor, guanidinoacetate, are primarily synthesized in the liver and kidneys and then transported to the brain. The creatine concentration calculated from the Cr MRS signal is about 9 mmol/L. However, as Cr has an important buffer capacity in cellular energy metabolism, its concentration cannot be considered 100% stable; cerebral levels can vary across different diseases or pathological states (Rosen and Lenkinski 2007; Ross and Bluml 2001). Nevertheless, using the Cr signal from a spectrum as an "internal concentration reference" when reporting metabolite levels from other resonances of the same spectrum (e.g., NAA/Cr, Cho/Cr) is experimentally straightforward (and corrects for some experimental factors when comparing measures between different individuals); this simple approach has at least in early reports proven to be of some diagnostic importance in many studies of various pathologies. Careful studies, however, have shown a brain activity-dependent change in Cr signal intensity (Ke et al. 2002), an age-dependent increase of Cr in WM (Maudsley et al. 2009), and changes of absolute creatine concentrations in various pathologies, so that Cr cannot be considered a useful concentration reference (Li et al. 2002; Ross and Michaelis 1994; Sartorius et al. 2008). The highest Cr levels are found in cerebellum, parallel to the distribution of creatine kinase and energy-requiring processes in the brain (Pouwels and Frahm 1998).

Glutamate is the major excitatory neurotransmitter in the human brain, mediator of synaptic plasticity and is implicated in the initiation and maintenance of addictive disorders (Kalivas and O'Brien 2008; Spanagel 2009). It is linked to metabolism through a neurotransmitter cycle between neurons and astrocytes. In this cycle, neurotransmitter/neuromodulator molecules released by the neurons are taken up by transporters in surrounding glial cells. In the glia, they are converted to glutamine which is released to the neuron, where it is used for the re-synthesis of the neurotransmitter. Glu is less concentrated in cortical WM than in GM (Pouwels and Frahm 1998). Although Glu is present in the brain at even higher concentration than NAA (about 11 mmol/L in GM), the MR detection sensitivity is poor due to the Glu signal being spread over a large number of closely spaced multiplett resonances, due to cancellation of overlapping resonances secondary to phase differences at longer echo times often used for MRS, and due to spectral overlap with equally complicated glutamine resonances. With the increasing availability of 3 T and higher magnetic field strength instruments, acquisition schemes that simplify the Glu resonance signal, and spectral fitting routines that make use of detailed resonance information, the quantification of cerebral Glu has become increasingly feasible.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain with a concentration of approximately 1.2 mmol/L in normal human cortex for the entire metabolically active pool (Hetherington et al. 1998; Rothman et al. 1993a). Abnormalities of GABA ergic neurotransmission have been implicated in many disorders, including alcoholism and in neurologic and psychological diseases such as epilepsy, Huntington's disease, depression and schizophrenia. Due to its lower concentration, its complicated MR spectral pattern, and due to its co-resonance with much larger metabolite signals (especially Cr), in vivo GABA quantitation is even more challenging than in vivo Glu quantitation. Special spectral editing methods are needed, the most frequently used of which is based on J modulation inhibition (Rothman et al. 1993b). GABA also co-resonates with unspecific macromolecules and it has been shown that 40–60% of the edited "GABA resonance" may come from macromolecules (Choi et al. 2007). Recent preliminary studies showed that the macromolecules are evenly distributed throughout the brain, supporting the assumption that changes observed in the edited "GABA resonance" are largely attributable to GABA changes (Xin et al. 2010). The potential, however, for inter-individual variability of the macromolecule contribution, especially in AUDs that are known to be associated with membrane alterations, needs to be investigated.

4 The Neurobiological Consequences of AUD and Changes During Abstinence from Alcohol

Since the early 1990s MRS in both humans and animals has been used to investigate the consequences of chronic and excessive alcohol consumption on brain metabolism. Most published studies examined (mostly alcohol-dependent)

Table 1 ^{1}H MRS studies performed in animal models of alcohol dependence

Reference	Animal model	Experimental design	Main findings
Hirakawa et al. 1994	Rats, single dose of ethanol	Field strength and method unknown	Low NAA 4 h after ethanol exposure
Braunová et al. 2000	Rats, 20% ethanol ad libidum for 8 and 14 weeks (mean 3.2 g/kg/day)	4.7 T, 16 ms Steam	Low mI/Cr after 8 weeks of ethanol diet
Lee H et al. 2003b	Rats 20% ethanol in drinking water for 16 and 60 weeks	4.8 T, Steam 68 ms	High Cho/NAA at week 16, low at week 60
Pfefferbaum et al. 2007	Rats, alcohol-preferring (50th generation) and thiamine deficiency and pyrithiamine administration	3 T GE whole body, J-resolved MRS	No primary effect or interaction of ethanol
Nicholas et al. 2008	Rats, single dose of ethanol (5 g/kg)	17 T, ex vivo (perchloric acid extracts)	No changes in brain metabolites
O'Leary-Moore et al. 2008	Rats, binge like-neonatal ethanol exposure	11.7 T, high resolution MRS ex vivo	Low NAA and taurine in cerebellum and striatum, high mI in cerebellum; low Glu in female rats; low Cho/Cr in hippocampus, high in striatum.
Zahr et al. 2008	Rats, 24 weeks of vaporized ethanol (14 h at night)	3 T, baseline, at 16 and 24 weeks	Cho increases after 16 and 24 weeks, mI, Glx and Glu increase after 24 weeks; trends for lower NAA and lower Cr
Zahr et al. 2010	Rats, binge model: 5 g/kg ethanol via oral gavage, then a maximum of 3 g/kg every 8 h for 4 days	3 T, baseline, 4 days binge, 7 days recovery; dorsal hippocampus	NAA and Cr decrease and Cho increases after 4 days; normalization after 7 days of abstinence
Weber-Fahr et al. 2010	Rats, chronic intermittent ethanol vapor intoxication	9.4 T, mPFC and hippocampus	Low mI and NAA, high Cho and Glu during intoxication; further increased Glu in early withdrawal

individuals in various phases of AUD treatment, whereas only a few studies examined the larger pool of chronically drinking treatment-naïve individuals. Duration and level of alcohol consumption, age, nutritional status (including plasma thiamine levels), family history of AUD and comorbid psychiatric and substance use disorders likely affect the magnitude and nature of brain metabolite abnormalities associated with chronic alcohol consumption. Furthermore, duration of abstinence from alcohol critically affects metabolite levels. Table 1 presents all peer-reviewed ^{1}H MRS studies performed in animal models of alcohol dependence, whereas Table 2 lists peer-reviewed ^{1}H MRS single-volume and ^{1}H MRSI multi-volume studies in humans.

The first MR study to suggest neuronal injury in the frontal cortex of abstinent alcoholics employed ^{1}H MRSI (Fein et al. 1994). Subsequent research has generally found reduced NAA levels in several brain regions of detoxified alcohol-dependent individuals, suggesting relatively widespread neuronal injury. Most single-volume ^{1}H MRS studies measured metabolites primarily in the frontal lobes and cerebellum of recovering alcoholics, brain regions most vulnerable to alcohol-induced tissue injury, and usually within 3–40 days of sobriety. They reported low NAA in the frontal lobes (Bendszus et al. 2001; Jagannathan et al. 1996), thalamus (Jagannathan et al. 1996) and cerebellum (Parks et al. 2002; Seitz et al. 1999) of individuals with AUD suggesting neuronal injury, atrophied dendrites and/or axons or derangement of metabolism. Other single-volume MRS studies reported lower cerebellar Cho (Bendszus et al. 2001; Parks et al. 2002) and elevated thalamic mI (Schweinsburg et al. 2000) relative to light-drinking controls, suggesting altered cell membrane metabolism and astrogliosis or osmotic changes. Lower concentrations of NAA in frontal WM and of NAA, Cho, and mI in the cerebellum correlated with lower neurocognitive and motor functioning (e.g., Bendszus et al. 2001; Parks et al. 2002). Higher Cho/Cr was reported in the occipital lobe of alcohol dependent patients (Modi et al. 2009), due to high Cho, low Cr concentrations or both. However, none of these human studies reported on significantly altered Cr concentrations in abstinent alcoholics.

Animal ^{1}H MRS studies of the direct effects of alcohol on brain metabolism have exclusively investigated rats. There are studies of the effects of single doses of ethanol (Hirakawa et al. 1994; Nicholas et al. 2008), rats bred for alcohol preference (Pfefferbaum et al. 2007), rats given 20% ethanol in water with ad libitum for at least 8 weeks (Braunova et al. 2000; Lee et al. 2003a), binge drinking models by oral gavage (Zahr et al. 2010) and chronic ethanol exposure in vapor chambers (Weber-Fahr et al. 2010; Zahr et al. 2009). The first rodent MRS study by Hirakawa (1994) reported decreased NAA following a single dose of ethanol. This initial finding was not replicated by other studies investigating single dose alcohol effects (Nicholas et al. 2008) or ad libitum ethanol consumption (Braunova et al. 2000) in non-selected rat populations or in genetically alcohol-preferring P rats (Pfefferbaum et al. 2007). Recent rodent MRS studies of 4 days of binge exposure (Zahr et al. 2010) and chronic intermittent alcohol vapor exposure (Weber-Fahr et al. 2010) indicate that excessive alcohol intake is causally inked to decreased NAA. Both studies found decreased NAA in intoxicated animals as well as a rapid recovery of

Table 2 ^{1}H MRS and MRSI studies performed in humans with alcohol use disorders (AUD)

Reference	Study groups	Duration of abstinence	Major findings (all findings statistically significant for patients compared to controls unless stated otherwise)
Fein et al. 1994	11A/9C	3–24 months	Greater frontal than parietal cortical NAA loss
Martin et al. 1995	10A/9C	0–28 days	Increase of Cho/NAA in cerebellar vermis with abstinence
Jagannathan et al. 1996	10A/27C	1 month	Low NAA/Cr and NAA/Cho in cerebellum, frontal lobe and thalamus
Seitz et al. 1999	11A/12C	4 days	Low NAA/Cr at TE = 135 ms and low Cho/Cr at TE = 5 ms
Behar et al. 1999	5A/10C	5 weeks	Low GABA + homocarnosine in occipital lobe
Schweinsburg et al. 2000	4A/5C	5 weeks and 6 years	High mI in anterior cingulate cortex and thalamus. NAA, Cho and Cr not altered. mI normal at 6 years.
Schweinsburg et al. 2001	37A/15C	1 month	Low NAA in frontal WM, high mI in WM
Bendszus et al. 2001	17A/12C	2 days and 5 weeks	Low NAA/Cr in cerebellum and frontal lobe, low Cho/Cr in cerebellum (days 3–6) and normalized values after 5 weeks
Parks et al. 2002	31A/12C	4 days and 3 months	Low cerebellar NAA and Cho. NAA increase over 3 months ($n = 11$).
Durazzo et al. 2004	24A/26C	1 week	Low NAA and Cho in frontal lobe, low Cho in thalamus and parietal lobe. Low NAA throughout brain of smoking vs. non-smoking alcoholics. Cr and mI unchanged.
Mason et al. 2006	12A/8C	1 week and 1 month	No GABA group differences in occipital gray matter, but higher GABA in non-smoking versus smoking patients. High glutamate + glutamine in smokers. GABA decrease in non-smoking alcoholics over 1 month.
Durazzo et al. 2006a	25A/29C	1 and 4 weeks	NAA and Cho increase in frontal and parietal lobes over 4 weeks. mI and Cr in frontal white matter increase over 4 weeks. Changes more pronounced in non-smoking alcoholics.
Ende et al. 2005	33A/30C	3 weeks, 3 and 6 months	Low Cho in cerebellum and frontal lobe, low NAA in frontal WM; Cho increases over 3 months ($n = 14$); no longitudinal NAA changes over 3 and 6 months ($n = 11$)
Bartsch et al. 2007	15A/10C	5 days and 6 weeks	Low frontomesial NAA and low cerebellar Cho. Both increase over 6–7 weeks

(continued)

Table 2 (continued)

Reference	Study groups	Duration of abstinence	Major findings(all findings statistically significant for patients compared to controls unless stated otherwise)
Durazzo et al. 2008	70A	1 month	Low NAA in temporal gray matter and frontal white matter and low Cho in frontal gray matter predict relapse within 6–12 months after treatment
Gazdzinski et al. 2008a	35A/ 32HD	1 week (alcoholics)	Low NAA, Cho, and mI throughout the brain of treatment-seeking alcoholics versus non-treated heavy drinkers
Gazdzinski et al. 2008b	24A/ 14C	1 week and 4 weeks	Low NAA and Cho in medial temporal lobe. NAA and Cho increase in non-smoking alcoholics over 4 weeks.
Modi et al. 2009	9A/13C	1 week	High Cho/Cr in occipital lobe
Durazzo et al. 2010b	51A/ 26C	1 week	Low NAA and Cr in brain reward system of those who relapse within 6–12 months after treatment versus abstainers and controls
Umhau et al. 2010	33A	days 4 and 25 of medication	Longitudinal decrease of Glu/Cr in anterior cingulate cortex of those treated with acamprosate ($N = 15$) versus placebo ($N = 18$)

A = alcohol dependent patients, C = control subjects, H = heavy drinkers

NAA with discontinuation of alcohol exposure. Zahr et al. (2010) found normal NAA levels after 7 days of recovery and Weber-Fahr et al. (2010) reported normal levels within 12 h of abstinence in the chronically exposed rats.

Abstinence from alcohol is associated with variable levels of recovery from chronic alcohol-induced brain volume loss (atrophy) in humans (e.g., Pfefferbaum et al. 1995). Longitudinal ^{1}H MRS studies during abstinence have the potential to illuminate some of the basic metabolic/cellular processes underlying such volume recovery. Again, early studies focused primarily on the frontal lobes and cerebellum. Martin et al. (1995) observed increased Cho/NAA in the cerebellar vermis over 3–4 weeks of abstinence from alcohol. Bendszus et al. (2001) reported increases in both frontal and cerebellar lobar NAA/Cr and cerebellar lobar Cho/Cr ratios after approximately 5 weeks of abstinence. After that interval, a higher frontal NAA/Cr ratio was related to better auditory-verbal memory while increased cerebellar vermis NAA/Cr ratio positively correlated with attention/concentration. Parks et al. (2002) observed that vermis NAA levels increased over 3 months of abstinence from alcohol, which was also related to improved auditory-verbal learning. In contrast to the group's earlier study (Martin et al. 1995), vermian Cho levels did not recover after 3 months, and the authors suggested this might indicate continued compromise of cerebellar vermis tissue, consistent with neuropathologic findings (Harper 1998). Higher mI was observed in the anterior cingulate gyrus, thalamus, frontal and parietal WM of 1 month-abstinent alcoholics but not in 6 year-abstinent alcoholics (Schweinsburg et al. 2001, 2000), suggesting reversible membrane breakdown or osmolytic changes with abstinence from alcohol. Bartsch et al. (2007) reported significant increases of cerebellar Cho and mesial frontal NAA over approximately 1 month of abstinence. Increasing mesial frontal NAA was positively related to improving attention. Of note, the authors included only smoking alcoholics who consumed less than 10 cigarettes per day. In a longitudinal multi-volume ^{1}H MRSI study, Ende et al. (2005) observed decreased Cho concentrations in the frontal WM, dorsolateral prefrontal cortex, superior frontal gyrus and cerebellar GM and vermis in individuals 1–4 weeks after detoxification and a significant Cho recovery over the following 3 months of abstinence. Decreased NAA was observed to be relatively persistent in the frontal WM, as no metabolite recovery (besides Cho) was observed after 3 months and no further recovery of any of the metabolite levels were apparent between 3 and 6 months of abstinence.

Ende et al. (2006), (2010) also found that higher alcohol consumption in non-abstinent individuals with AUD and in light-drinking controls without AUD was associated with higher frontal Cho levels. While associations between higher Cho and more acute alcohol consumption have been corroborated in rodent studies (Lee et al. 2003b; Weber-Fahr et al. 2010; Zahr et al. 2010; Zahr and Sullivan 2008), human studies in recently detoxified and 1 month-abstinent alcoholics have found unchanged or reduced Cho levels (Schweinsburg et al. 2001, 2000; Bendszus et al. 2001; Durazzo et al. 2004; Ende et al. 2005; Parks et al. 2002). Additionally, Cho was reported to show signficant increases or normalize with continued abstinence (Durazzo et al. 2006a; Ende et al. 2005). Whether this difference is a consequence

of chronic and excessive alcohol consumption or a consequence of detoxification per se requires further investigation. Zahr et al. (2010) suggested that low Cho in human studies can be explained by undetected or subclinical pathologies, including thiamine deficiency or liver cirrhosis. Lee et al. (2003a) found an initial Cho increase in rats after 16 weeks of alcohol exposure followed by a significant decrease after 60 weeks, which supports the hypothesis that Cho levels reverse and decrease below normal with duration of chronic alcohol abuse. More recent rodent studies, however, failed to detect a significant Cho decrease with prolonged exposure or during withdrawal (Weber-Fahr et al. 2010; Zahr et al. 2010, 2009). Therefore, the time course of Cho concentration changes in alcohol dependence and during abstinence do not appear to be consistent across studies, and the significance of the temporal Cho level dynamics as a function of drinking severity are as of yet unclear. They are likely complicated by the fact that Cho levels reflect different biological processes, including cellular membrane turnover and density as well as myelin anabolism and catabolism.

To date, there is no fully quantitative AUD study that reported significant reductions in Cr concentrations relative to light-drinking controls and only one study (Durazzo et al. 2006a) that reported a significant Cr increase with abstinence. In contrast, recent rodent studies of active alcohol exposure support a Cr decrease (Zahr et al. 2010; Weber-Fahr et al. 2010) that quickly normalized after alcohol withdrawal. Furthermore, mI, the putative astrocyte marker and osmolyte, decreased in rodents during chronic ethanol exposure and recovered to normal levels after alcohol withdrawal (Braunova et al. 2000; Weber-Fahr et al. 2010). Studies in human alcoholics reported no mI changes, except for one study showing elevated mI in some brain regions after detoxification (Schweinsburg et al. 2000). Although not consistent across species, the mI findings overall suggest some alcohol-related alterations of cell membrane metabolism and gliotic or osmotic changes.

Modulations and adaptations of reciprocal glutamatergic and GABAergic projections from frontal brain regions, basal forebrain and midbrain likely contribute to the neural basis of substance dependence (Kalivas and Volkow 2005). Chronic alcohol-induced adaptations within the glutamatergic systems, a hyperglutamatergic state, contribute to the induction and maintenance of alcohol dependence (Kalivas et al. 2009; Spanagel 2009). Those adaptations then may cause a hyper-excitability of the central nervous system when alcohol is removed (withdrawal) and represent a mechanism involved in early relapse behavior (Littleton 1995). This concept receives support from the application of glutamate modulators or functional glutamate antagonists, such as acamprosate, for the reduction of alcohol consumption. Such pharmacotherapies have become increasingly important in treating both AUD and other substance use disorders, centering on medications modulating common neurotransmitters such as serotonin, dopamine, Glu and GABA. Thus, a better understanding of the specific effects of AUD on brain GABA and Glu concentrations and flux may further advance the development and efficacy of pharmacological treatment. Basal cerebral concentrations of specific neurotransmitters have been linked to behavior. In rats,

frontal Glu transmission has been associated with drug seeking (Kalivas and Volkow 2005; McFarland et al. 2003), and in humans, low striatal Glu concentration measured by ^{1}H MRS has been linked to a decline in neurocognitive test performance with normal ageing (Zahr et al. 2008). Glu concentrations in the prefrontal medial cortex were associated negatively with sensation seeking and positively with measures of impulsivity (Gallinat et al. 2007a; Hoerst et al. 2010). Modulation of the inhibitory GABA system by alcohol is implicated in the development of alcohol tolerance, dependence and withdrawal and in emotional processing/judgment. In humans, some studies report decreased plasma and CSF GABA at 1 month of abstinence from alcohol and normal GABA levels by 6 months of sobriety (Adinoff et al. 1995; Coffman and Petty 1985).

Although the ^{1}H MRS detectable amino acid levels represent the metabolically available brain pools (which are much larger than the respective neurotransmitter pools), they are in tight equilibrium with synaptic levels (Rothman et al. 2003). Therefore, MRS-derived GABA and Glu concentrations provide valuable information on the role and functional significance of these neurotransmitter systems. Consistent with plasma and CSF GABA levels, tissue GABA levels measured by ^{1}H MRS in occipital cortex of a small sample of alcoholics at about 1 month of abstinence were approximately 25% lower than in non-alcoholic controls (Behar et al. 1999). A later study, however, showed that GABA levels were elevated in 1 week abstinent non-smoking alcoholics and normalized after 4 weeks (Mason et al. 2006). GABA levels in smoking alcoholics were normal and did not change over time. Glu is an endogenous agonist of N-methyl-D-aspartate receptors and their increased activity (postsynaptic receptors) may produce neurotoxicity presumably through dysregulation of Ca2 + influx (see Bleich et al. 2004; De Witte 2004). Glu levels are increased during alcohol withdrawal in animal models (see Bleich et al. 2004 for review) and in anterior cingulate cortex of humans (Frischknecht et al. 2010). At 6 days of abstinence, anterior cingulate Glu was decreased below control levels and then increased significantly into the control range within the following 24 days of sobriety (Mon et al. 2010). An early in vivo ^{1}H MRS study suggests that Glx (the sum of Glu and glutamine) in healthy controls is lower relative to placebo 20 min after infusion of acamprosate (which has been shown to decrease alcohol consumption) (Bolo et al. 1998), consistent with microdialysis results in alcohol-dependent rats treated with acamprosate (Dahchour et al. 2005). In a small placebo controlled clinical trial, Glu in anterior cingulate (relative to Cr levels) decreased in recently abstinent alcohol-dependent individuals over 4 weeks of treatment with acamprosate (Umhau et al. 2010). In rats, Zahr et al. (2009) found increased Glx after 24 weeks of ethanol vapor exposure when the blood alcohol level was still high, while Weber-Fahr et al. (2010) detected increasing Glu levels during acute withdrawal that were still high after 3 days of abstinence. Glu levels normalized within 3 weeks of abstinence when withdrawal symptoms subsided. Together, these dynamic changes observed in both rodent and human studies are consistent with a hyperglutamatergic state during withdrawal and normalization of central Glu levels within a few weeks of sustained abstinence.

5 Treatment-Seeking Versus Treatment Naïve Alcoholics and Common Comorbidities

The vast majority of research investigating brain changes in AUD has been conducted with individuals in substance abuse treatment. Although readily accessible for scientific research, these treatment-seeking individuals are a minority among persons with AUD, with the majority being treatment-naïve (Fein and Landman 2005; Hasin et al. 2007). Treatment-seeking cohorts also generally present with a higher severity of medical, psychiatric and substance use comorbidities that may affect MR outcome measures. These common comorbidities may independently influence brain biochemistry, structure and function. Furthermore, treatment-seeking alcoholics usually have more severe alcohol consumption (up to 50% higher consumption over lifetime) and more periods of abstinence than their treatment-naïve counterparts (Fein and Landman 2005). Due to the prevalence and/or magnitude of comorbid conditions and alcohol consumption, it may be reasonably expected that the treatment–seeking population has generally greater brain injury than the treatment-naïve population and that both do not simply represent a continuum of AUD on a progressive scale. It is widely recognized that these comorbid characteristics may promote considerable variability in the pattern and magnitude of neurobiological and neurocognitive abnormalities demonstrated in AUD, during detoxification and sustained abstinence. Our research has focused on studying the unique impact of common comorbidities on the brain in persons with AUD to try to better understand the brain changes in this complex population (reviewed in (Durazzo and Meyerhoff 2007).

In a ^{1}H MRSI study we observed that, relative to light-drinking controls, metabolite abnormalities in community-dwelling heavy drinkers (the vast majority were alcohol-dependent) are less pronounced and demonstrate a different pattern of metabolite abnormalities than reported in recently abstinent treated alcoholics (Gazdzinski et al. 2008a; Meyerhoff et al. 2004). Nevertheless, compared to light/non-drinking controls, treatment-naïve heavy drinkers had lower NAA concentrations in frontal WM and parietal GM, suggesting greater neuronal injury, and parietal GM Cr was elevated. Although small, the frontal NAA reduction was functionally significant as it was associated with poorer performances on measures of executive skills and working memory as well as lower frontal P300b amplitudes. Furthermore, age, sex, family history of alcohol problems and drinking pattern (binge vs. non-binge) modulated brain metabolite abnormalities. We also compared these treatment-naïve heavy drinkers to 1 week-abstinent treatment-seeking alcoholics (Gazdzinski et al. 2008a). In conjunction with smaller lobar GM volumes and thalami in treatment-seeking versus treatment-naïve individuals, NAA, Cho and mI concentrations were lower in multiple brain regions. While lower WM NAA was completely explained by average number of drinks per month over 1 year prior to study, the other metabolite group differences were not explained by alcohol consumption levels, demographic, and clinical variables or by psychiatric comorbidities.

Taken together, the brain structural, metabolic and functional differences between treatment-seeking and treatment-naïve alcoholic populations suggest that the neurobiological abnormalities observed in clinical convenience samples of alcoholics in treatment cannot be generalized to the much larger treatment-naïve population with AUD. Instead, the comorbid occurrence of neuropsychiatric and substance use factors need to be considered specifically when examining neurobiological and neurocognitive consequences in AUD.

The most prevalent comorbidity in AUD is chronic cigarette smoking. It is estimated that 60–80% of AUDs are chronic smokers (Durazzo et al. 2007; Romberger and Grant 2004). A growing body of research suggests that chronic smoking, independent of AUD, is associated with abnormalities in brain morphology, cerebral blood flow, neurochemistry and neurocognition that are similar to those reported in AUD (Durazzo and Meyerhoff 2007). We have investigated the effects of concurrent chronic cigarette smoking on regional brain morphology (Gazdzinski et al. 2005), blood flow (Gazdzinski et al. 2006; Mon et al. 2009) and metabolite concentrations (Durazzo et al. 2004) in 1 week-abstinent, treatment-seeking individuals with AUD as well as longitudinal brain metabolite changes during short-term abstinence from alcohol (Durazzo et al. 2006a). Both chronic alcohol consumption and chronic smoking independently are associated with significant neocortical GM loss. We observed that 1 week-abstinent, treatment-seeking smoking alcoholics compared to their non-smoking counterparts demonstrated lower NAA concentrations in frontal WM, parietal GM, and lenticular nuclei as well as lower NAA and Cho in the midbrain (Durazzo et al. 2004). Alcohol dependence, independent of smoking, was associated with lower Cho concentrations in the thalamic and parietal lobes and lower frontal lobe NAA and Cho, the latter consistent with other reports (see above). Neither alcohol dependence nor chronic smoking was associated with significant alterations of Cr and mI concentrations in any of the lobar regions analyzed. Among smoking alcoholics, greater nicotine dependence and a higher number of cigarettes per day were negatively correlated with absolute NAA concentrations in thalamic and lenticular nuclei. Lower cerebellar vermis NAA was associated with poorer visuomotor scanning speed (smokers) and poorer visuospatial learning and memory (non-smokers). These in vivo ^{1}H MRSI findings suggest that chronic smoking compounds alcohol-induced neuronal injury and cell membrane injury in the frontal lobes of persons with AUD and has independent adverse effects on neuronal viability and cell membrane turnover/synthesis in the vermis and midbrain. Findings are largely consistent with metabolic changes described in non-alcoholic chronic smokers (Gallinat et al. 2007b). ^{1}H MRS studies also showed that brain GABA concentrations in animals and humans are modulated by nicotine and/or cigarette smoking (Epperson et al. 2005; Zhu and Chiappinelli 1999), so that alterations of these metabolites in smoking alcoholics would not be a surprise. Such smoking-induced metabolic brain abnormalities are likely of clinical significance as they are accompanied by lower performance on cognitive tests that require fast and flexible processing, such as set-shifting, processing speed and cognitive efficiency (see e.g., Durazzo et al. 2006b; Friend et al. 2005; Glass et al. 2006 and references cited therein).

In longitudinal ^{1}H MRSI studies of treatment-seeking alcoholics, after approximately 1 month of abstinence from alcohol, we found significant increases of NAA and Cho concentrations in WM and GM of the frontal and parietal lobes (Durazzo et al. 2006a), consistent with the literature described above (Bendszus et al. 2001; Ende et al. 2005). Despite not being significantly reduced cross-sectionally, mI and Cr levels increased over time in the frontal WM only. When smoking status was considered, non-smokers showed widespread metabolite increases, whereas increases in smokers were much less pronounced and seen in fewer brain regions. In non-smokers, metabolite gains were related to improvements in visuospatial learning, visuospatial memory and working memory, visuomotor scanning speed and incidental learning, while smoking alcoholics showed significantly fewer of such relationships. Furthermore, in abstinent smokers, longer smoking duration was related to smaller longitudinal increases in frontal WM NAA, frontal WM Cho, and thalamic Cho. Similarly, NAA and Cho levels increased significantly in medial temporal lobe (including hippocampal tissue) of non-smokers abstinent for 1 month, but the corresponding concentrations in smokers remained depressed relative to non-smoking light-drinking controls (Gazdzinski et al. 2008b). mI tended to increase in non-smoking alcoholics. In the combined alcoholic cohort (i.e., smokers plus non-smokers), increasing Cho, Cr and mI were associated with improving visuospatial memory.

Occipital GM GABA concentrations during recovery from AUD are also modulated by smoking status (Mason et al. 2006). At 1 week of abstinence, cortical GABA levels were higher in alcohol-dependent non-smokers than smokers. After approximately 3 weeks of abstinence, GABA levels were lower than at 1 week and similar between alcoholic non-smokers and smokers. Higher GABA during early withdrawal may reflect compensation for reduced cortical benzodiazepine-$GABA_A$ receptor function thought to contribute to alcohol tolerance and withdrawal. The subsequent decline may reflect "normalization" of $GABA_A$ receptor function with sobriety.

6 The Neurobiological Correlates of Relapse in Alcohol Use Disorders (AUD)

More than 60% of individuals who seek treatment for AUD will return to hazardous levels of alcohol consumption, with the majority relapsing within 6 months following their last treatment (see Durazzo et al. 2010a, 2010b). While much research has addressed the potential neuropsychological, psychiatric, sociodemographic and behavioral factors associated with relapse in AUD, the neurobiological factors associated with sustained sobriety and/or increased risk for relapse after treatment for AUD are not well understood. A greater understanding of these objective factors can provide a better understanding of the mechanisms driving the relapse/remit cycle and maintenance of long-term abstinence.

In a longitudinal ^{1}H MRS study of treatment-seeking alcohol-dependent individuals, Parks and colleagues (Parks et al. 2002) observed that those who relapsed within 3 weeks of study demonstrated lower cerebellar NAA and Cho at 3–5 days of abstinence relative to controls. However, cerebellar or frontal metabolite concentration reductions were not observed between controls and individuals who relapsed after 3 weeks of abstinence. A more recent study combined ^{1}H MRSI measurement of absolute regional metabolite concentrations with assessment of major psychiatric disorders, and comprehensive neurocognitive testing in treatment-seeking participants at approximately 1 month of abstinence from alcohol (Durazzo et al. 2008). Participants were followed for 6–12 months after treatment, and were retrospectively classified as abstainers (no alcohol consumption) and resumers (any alcohol consumption) and then contrasted on outcome measures at 1 month of abstinence. Temporal GM NAA, frontal WM NAA, frontal GM Cho, processing speed and comorbid unipolar mood disorder were independent predictors of resumption of drinking. In a companion ^{1}H MRSI study of a largely similar population, we analyzed metabolite levels at 1 week of abstinence in regions of the extended brain reward system as a function of relapse status (Durazzo et al. 2010b). Resumers demonstrated significantly lower baseline NAA concentrations than non-smoking non/light-drinkers and abstainers in dorsolateral prefrontal cortex, anterior cingulate cortex, insula, superior corona radiata and cerebellar vermis. Resumers also exhibited lower Cr concentrations than abstainers in the dorsolateral prefrontal cortex, superior corona radiata and cerebellar vermis. Importantly, abstainers did not differ significantly from the controls on baseline metabolite concentrations in any region. In resumers, moderate to strong relationships were apparent between regional baseline NAA levels and several measures of post-treatment alcohol consumption, strongly suggesting that lower neuronal integrity in regions of the extended brain reward system at baseline predicts greater relapse severity.

7 Conclusions and Perspectives

This review describes the association of AUD with adverse neurobiological consequences as measured by ^{1}H MRS in treatment-seeking and non-treatment-seeking cohorts and animal models. The most consistent finding is reduced NAA in both AUD and chronically alcohol exposed rats and its partial (human) to full (rat) reversibility with sustained abstinence. In general, alcohol-induced changes in animal brain metabolites are seen during the intoxication phase, with little if any long-lasting dependence-related changes in brain metabolites. Most human studies, however, not only detect neurobiological changes after years of chronic alcohol consumption, but also observe partial recovery of NAA over several months of abstinence, while Cho ultimately recovers to control levels within a shorter time frame. The potential of ^{1}H MRS to monitor dynamic changes of metabolite levels as a function of duration of sobriety and as a consequence of pharmacological treatment has been demonstrated in both animals and humans.

A review of the human AUD literature suggests that examining individuals with AUD as a homogeneous group without consideration of common comorbidities, such as other substance use (including chronic cigarette smoking) and psychiatric disorders (even if subclinical) may confound our understanding of the factors contributing to the neurobiological and neurocognitive dysfunction observed in AUD. Additionally, failure to consider the potential influence of common comorbidities in AUD may obscure the identification of the neurobiological factors associated with neurobiological recovery during abstinence as well as those related to relapse. MR-based spectroscopy studies of animal models have the advantage of investigating the nature and pattern of brain injury that are specifically related to chronic alcohol exposure, without the potential of confounding effects from comorbidities common in human AUD. To date, there are no ^{1}H MRS studies in animals that involved exposure to alcohol and other substances, for example the combined effects of alcohol and cigarette smoke/nicotine exposure on brain metabolite. In AUD, the comorbid misuse of other substances such as marijuana, cocaine and cigarettes is exceedingly common (polysubstance use) and the clinical reality facing most treatment providers. Therefore, it may be advisable that future research with animal models incorporate other substances that are prevalent in AUD to facilitate increased generalizability of research findings to humans.

Human studies reveal that chronic smoking in treatment-seeking and treatment-naïve cohorts with AUD compounds regional neurobiological abnormalities. Furthermore, chronic smoking in AUD is associated with diminished, perhaps delayed, recuperation of regional biochemical markers of neuronal viability and cell membrane synthesis/turnover during abstinence from alcohol. If chronic cigarette smoking is confirmed to modulate brain neurobiology and neurocognition in additional human studies and in animal models, we may have to entertain the possibility that smoking and non-smoking individuals with AUD differ in the nature or extent of their response to pharmacological and/or behavioral interventions designed to promote abstinence from alcohol. Even without these confirmatory studies, the reviewed literature, in conjunction with the known mortality and morbidity associated with chronic smoking, lends support to the growing clinical initiative that encourages chronic smokers entering treatment for AUD to participate also in a smoking cessation program.

Given the high prevalence of medical, psychiatric and substance misuse comorbidities in AUD, it is important to understand to what extent these factors can contribute to the neurobiological and neurocognitive abnormalities observed in AUD. Examining AUD samples under particular consideration of these common comorbidities increases the clinical relevance and generalizability of the data, as such cohorts are more representative of the typical treatment-naïve and treatment-seeking populations. Additional prospective research, with larger groups of female participants is required to evaluate for sex effects, particularly since it is unclear if males and females manifest the same degree or pattern of alcohol-induced neurobiological and neurocognitive abnormalities at equivalent drinking severity levels (Mann et al. 1992; Parsons and Nixon 1998; Sullivan et al. 2004).

Moderate to strong relationships between ^{1}H MRS measures and various measures of neurocognition in cross-sectional and longitudinal studies indicate that MRS-derived neurobiological measures are robust and relevant predictors of brain function and therefore drug use behavior. Hence, the application of MRS in the study of neurobiological factors associated with abstinence and relapse highlight the clinical usefulness of this approach for the prediction of relapse, in conjunction with the more conventional neurocognitive and psychiatric factors. Recent ^{1}H MRS data support the potential of MR-derived measures to assist in the identification of objective factors associated with increased risk for resumption of hazardous drinking following treatment for AUD. Neuroimaging-based investigations of the factors associated with the chronic relapse/remit cycle in AUD may also facilitate the development of more efficacious pharmacological and behavioral interventions for AUD.

Finally, in vivo ^{1}H MRS, as part of the emerging field of "imaging genetics" may provide readily accessible, objective, functionally significant and region-specific neurobiological measures (endophenotypes) that successfully link specific genotypes to neurocognition and psychiatric symptomatology in relatively small patient cohorts (for review see Meyerhoff and Durazzo 2008). If early evidence of genetic effects on MRS-detectable metabolite measures are confirmed, MRS genetics research will not only offer clues to the functional significance of genetic differences in AUD, but MRS can potentially influence the future of clinical management of AUD via monitoring the efficacy of pharmacological and behavioral interventions as a function of genotype.

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Part V
Translational Aspects and Medication Developement

Translational Approaches to Medication Development

Selena Bartlett and Markus Heilig

Abstract Alcohol accounts for major disability worldwide and available treatments are insufficient. A massive growth in the area of addiction neuroscience over the last several decades has not resulted in a corresponding expansion of treatment options available to patients. In this chapter, we describe our experience with building translational research programs aimed at developing novel pharmacotherapies for alcoholism. The narrative is based on experience and considerations made in the course of building these programs, and work on four mechanisms targeted by our libraries: cholinergic nicotine receptors, receptors for corticotropin-releasing hormone (CRH), neurokinin 1 (NK1) receptors for substance P (SP) and hypocretin/orexin receptors. Around this experience, we discuss issues we believe to be critical for successful translation of basic addiction neuroscience into treatments, and complementarities between academic and other actors that in our assessment need to be harnessed in order to bring treatments to the clinic.

Keywords Nicotinic · Varenicline · Corticotropin-releasing hormone · Substance P · Neurokinin · Orexin · Hypocretin

S. Bartlett (✉)
Preclinical Development Group, Ernest Gallo Clinic and Research Center, University of California San Francisco, 5858 Horton Street, Emeryville, CA 94608, USA
e-mail: SelenaB@gallo.ucsf.edu

M. Heilig
National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892-1108, USA

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Contents

1 Introduction 544
2 Overview of Translational Approaches for Medications Development 546
3 Novel Therapeutic Targets and Medications Arising from this Approach 549
3.1 Neuronal Nicotinic Receptors 549
3.2 Corticotropin-Releasing Hormone 556
3.3 Substance P and Its NK1 Receptor 562
3.4 Orexin Receptors 565
4 Personalized Medicine and the Promise of Pharmacogenetics 566
5 Final Comments 569
References 569

1 Introduction

In contrast to illicit drugs such as cocaine and heroin, alcohol is initially not a potent reinforcer, and the delay between its oral ingestion and psychotropic effects further weakens its reinforcing properties. Controlled social use of alcohol is therefore widespread in large parts of the world, and the vast majority of people who engage in such use do not go on to develop alcohol-related problems. Following mild intoxication, social drinkers tend to become relaxed and feel an improvement of mood, effects that are clearly considered desirable by many. However, in the absence of tolerance, sedative and ataxic properties of alcohol emerge at higher doses, and impose an inherent limit on the amounts that can be consumed. Some people are less sensitive to these CNS-depressant effects of alcohol, and their "low response" to alcohol is a heritable trait that is associated with an increased genetic susceptibility for alcohol dependence (hereafter equated with alcoholism) (Newlin and Thomson 1990). Innate tolerance of this kind, or tolerance that is acquired as an individual's drinking progresses, allow people to escalate their alcohol use. Over time, however, it also takes more and more alcohol to achieve the same high. Eventually, consumption escalates to heavy-drinking, the high is hardly present, a negative affective state emerges in the absence of alcohol, and the development of alcoholism ensues.

Alcohol use accounts for 4% of global disease burden, with a worldwide distribution that increases with affluence, indicating that a further rise is to be expected (Rehm et al. 2009). A medical approach to this problem remains controversial by some accounts, but is clearly warranted by the data. First, it is true that alcohol-related harm does not require the presence of alcoholism, and that the disease burden of alcohol is correlated with total consumption of alcohol in a population. Measures that limit total consumption are therefore also effective in reducing alcohol-related harm. However, the distribution of consumption is highly skewed. Typically, 10% of adults consume about half of all alcohol in a population, and incur the vast majority of alcohol-related harm (Rehm et al. 2003). Among this minority, a maladaptive pattern of heavy use is seen, the essential elements of which were captured in a classical account many years ago (Edwards and Gross 1976). Secondly, this maladaptive

pattern of heavy-drinking shares critical characteristics with established, common medical conditions, such as diabetes, asthma and hypertension. The similarities include a considerable component of genetic susceptibility, an important role of environmental exposure, a high degree of dependence on lifestyle and behavioral choices, and perhaps most importantly, a chronic relapsing course (McLellan et al. 2000). The differences are thus not between alcoholism and these established medical conditions, but rather between their management. For the latter, long-term disease management is considered the standard of care, and is based on integrating ever improving pharmacotherapies with strategies aimed at modifying behavior. For the former, anything but a "cure" is frequently considered a failure, and the role of pharmacotherapies remains disputed. Against this background, the way in which alcoholism is treated is in our view in need of a major change.

Despite its devastating impact on society, few effective medications are available for the treatment of alcoholism. Three medications have been approved for this indication by the US. Food and Drug Administration since 1940: disulfiram (Antabuse™), naltrexone (ReVia™, Vivitrol™) and acamprosate (Campral™). The opioid antagonist naltrexone is reported to effectively reduce ethanol consumption both in animals (Altshuler et al. 1980; Stromberg et al. 1998) and humans (Volpicelli et al. 1992; O'Malley et al. 1992), offering an elegant example of early translational work. Naltrexone has perhaps the most consistent effect in reducing alcohol consumption in combination with behavioral therapy (Anton et al. 2006). The discovery of naltrexone as a pharmacotherapy for alcoholism was a critical conceptual advance, because it effectively demonstrated that, contrary to widely held perceptions, meaningful clinical improvement can be achieved in alcoholism with the help of a medication, and by targeting a well-defined neurobiological mechanism. However, not all patients respond to naltrexone, and the average effect size is small (Bouza et al. 2004). Because of this and other factors, naltrexone is not widely prescribed (Mark et al. 2003), and alcoholism treatment largely continues to be delivered outside a medical setting, without the aid of pharmacotherapy.

Interestingly, and as discussed in detail below, the small average effect size of naltrexone is in fact likely to reflect a considerable degree of heterogeneity, in part explained by genetic variation at the locus encoding the target for this medication, the μ-opioid receptor (Bond et al. 1998; Oslin et al. 2003; Anton et al. 2008). This highlights the need for novel alcoholism treatments to be tailored, so that they take in account genetic and other differences between patients in the highly heterogeneous alcohol dependent category. A likely implication, further, that no medication will ever be a magic bullet, or perhaps a commercial blockbuster. However, as additional mechanisms become possible to target with development of new medications, overall outcomes will continue to improve.

Basic neuroscience research has identified numerous candidate targets for pharmacological treatment of alcoholism, but translation into clinical development has so far been limited (Heilig and Egli 2006). In the following, we analyze some of the reasons for this, and discuss our laboratories' strategies and experience with trying to break through this barrier.

2 Overview of Translational Approaches for Medications Development

The development and production of most successful pharmacological treatments has historically been carried out by the pharmaceutical industry. In part, this is because the drug development process requires the assembly of elements that are not easily put together in a grant funded academic environment, as discussed below. In part, however, this is also driven by factors external to the scientific process. Whether one likes it or not, once developed, for a medication to become widely used clinically, it needs to have the backing of a major pharmaceutical company, and rely on its sales force for dissemination in the clinical arena. For this to happen, in turn, there needs to be a commercial value, based on carefully managed intellectual property.

The fact that major pharmaceutical companies have historically not invested in developing compounds for alcoholism or other addictive disorders are therefore likely to be an important reason contributing to the paucity of alcoholism medications. Analyzing the barriers that have prevented this from happening is essential to changing the current situation. Three main categories of factors can be identified:

1. Alcoholism continues to carry a stigma. Related to this is a perception that individuals affected by this condition are largely uninsured, implying that new medications could not succeed commercially. Addressing this factor in detail is beyond the scope of the present chapter, but nevertheless merits a few comments. First, even if true, the reimbursement issue would only apply to one of the major world markets, the US. In contrast, in the larger EU market, any medication approved based on favorable efficacy and safety data would be equally paid for. Secondly, and perhaps more importantly, the vast majority of subjects with alcohol use disorders are in fact socially stable, earn incomes, and have insurance. Currently, they remain largely undiagnosed, and in the US, less than one in four subjects with outright alcohol dependence has ever been treated (Hasin et al. 2007). Thus, normal market analysis models simply do not apply in this case. At this early stage, the issue for a novel alcoholism medication is not to capture a share of a market, but rather to build this market.
2. Alcoholism is a complex and heterogeneous condition. Proposed target mechanisms for treatment are both too few and too many. A large number of candidate biological mechanisms have been proposed by academic laboratories as potential targets for alcoholism treatment. In the absence of industry involvement, however, few of these have been validated across a range of models, or extensively evaluated for safety and specificity, work that has few immediate rewards for an academic investigator. For the same reason, resources for chemical discovery, lead optimization, preclinical and human safety studies or early human translation have not been available to advance—or, equally important, put to rest–proposed candidates. Creating public—private partnerships that can bridge this gap and

lower the threshold for major pharma to engage is in our view critical for advancing treatment development for alcoholism.

3. Even after the factors above have been addressed, there is a valid concern among pharmaceutical companies that alcohol dependent patients have complex medical and behavioral morbidity, and that safety problems in this population could jeopardize the development of molecules that might otherwise be promising for other indications. Given the tremendous investment behind any clinical candidate molecule, this concern needs to be understood and managed. Doing so can include picking up good molecules that have been discontinued from development for other indications, or working with backup compounds once a lead compound has succeeded for another indication. An additional way of taking out the risk for companies from engaging, and creating opportunities for early human translation, is to carry out early human studies under highly controlled laboratory conditions where safety can be assured.

Against this background, we believe that academic centers can make key contributions in advancing novel treatments by building interactive multidisciplinary environments that combine a capacity for target identification and validation (or "target ID" for short) on one hand, with that for early human translation on the other. The challenge is obviously the broad spectrum of methodologies even this limited development capacity requires. Target ID requires state-of-the-art resources and expertise in the areas of molecular and cell biology, electrophysiology, animal behavior and genetics. The target ID teams need to be committed to the translation of their findings into human clinical research and drug development, and ultimately the transfer of this knowledge to patients. There are many considerations prior to attempting translation of basic research discoveries into novel therapeutics or treatment approaches. One of the most important issues to consider is the animal model that generated the target ID, how predictive the findings can be expected to be for the human condition, and how they can inform human studies e.g. with regard to choice of target populations and design. Fortunately, a range of animal models in rodents and non-human primates appears to have some degree of predictive validity with regard to suppression of alcohol intake (Egli 2005). This may reflect that at a fundamental level, the interaction of alcohol and other addictive drugs with the brain is similar enough across mammals (and perhaps beyond), while other neurological and psychiatric conditions may be considerably more complex, and may in some cases, such as schizophrenia, be unique to humans. The second issue is the likelihood that the candidate mechanisms being considered can be targeted, or the molecule being tested can be given to subjects for the treatment of addiction in a safe and effective manner. For instance, directly blocking the function of a key neurotransmitter system such as dopamine or glutamate might well be capable of suppressing ethanol intake, but would also be expected to influence a wide range of other behaviors. Finally, because drug effects on alcohol related behaviors in animals may also reflect activity in other important areas, such as anxiety and pain, target ID efforts for alcoholism may also yield new therapeutics for other CNS disorders.

Early human translation of results from the target ID process requires resources and expertise in the areas of clinical pharmacology, human behavioral studies, functional brain imaging and human genetics. These resources can conveniently be grouped under the label of “experimental medicine”. At this stage of the development process, the focus is on surrogate markers of efficacy, i.e., drug effects on behavior under laboratory conditions, brain responses to alcohol or other relevant stimuli, or other biomarkers. The key characteristics of these surrogate markers are that they can be obtained in the short term and under highly controlled conditions, can demonstrate target engagement at a particular dose level, and can be predictive of clinical efficacy. In a truly translational environment, the experimental medicine team interacts seamlessly and on a daily basis with the target ID team, so that prioritization of targets for, selection of subjects to, and design of the experimental medicine studies is continuously guided by insights from the target ID process.

In contrast, academic institutions do not currently have the resources to support a comprehensive therapeutic development program, which additionally requires medicinal chemistry, preclinical as well as clinical toxicology, and other downstream components such as execution of large multicenter trials that are essential for ultimately producing an FDA-approved medication. Therefore, partnerships with pharmaceutical or biotech companies that can contribute these resources are crucial in our view. How these partnerships are structured will vary on a case by case basis, and the process will in most cases be iterative.

In many cases, once a promising mechanism has been identified through academic efforts, access will be needed to candidate compounds that are the result of chemical discovery and optimization in industry. Although some efforts have tried to make this type of resources available to academic investigators, e.g. through the molecular libraries program (MLP) of the US National Institutes of Health (NIH), the massive capacity and expertise of the pharmaceutical industry in the area of medicinal chemistry can in our view not easily be duplicated. In a successful partnership, however, the academic lab will be able to contribute critical preclinical characterization of candidate molecules, increasingly required before a discussion of clinical development can be initiated. Next, in order to advance a promising candidate to a point where it can be evaluated using experimental medicine approaches, preclinical as well as clinical safety studies will have to be carried out. Once again, this is critical work that nevertheless does not thrive in an academic environment, because it has little innovation value, carries a cost that exceeds what can typically be funded by research grants, and does not generate a comparable publication output. Finally, if an efficacy signal is picked up in the course of experimental medicine evaluation, and is perhaps further supported by proof-of-concept data from a public mechanism such as the US National Institute on Alcohol Abuse and Alcoholism (NIAAA) Clinical Investigations Group (NCIG, www.getcontrol.org), advancing the clinical candidate to a new drug application (NDA) will require large multicenter trials sponsored by a drug company. Finally, as illustrated below, in some cases the target will be one for which therapeutics have already been developed and FDA-approved for a different indication, offering opportunities to explore their efficacy and safety as therapeutics for alcohol and substance abuse.

In summary, the barrier for academic institutions has historically been to translate basic science from the bench into targets of interest to the private sector of pharmaceutical and biotechnology companies, partners that have critically needed capabilities to develop medications for the treatment of alcoholism and other addictive disorders. This gap between basic research and human clinical studies is slowly being narrowed, as not-for-profit and academic institutions are applying innovative approaches to push bench-to-bedside programs for the development of novel therapeutics for addiction. Some of our experience from this type of efforts will be reviewed in the following.

3 Novel Therapeutic Targets and Medications Arising from this Approach

A social drinker typically has few problems with alcohol, no preoccupation with drinking, is able to control the amount of alcohol consumed, and rarely drinks to the point of pronounced intoxication. For these individuals, drinking is a secondary activity. It is the party, the meal, the wedding that interests the social drinker, not the opportunity to drink. At the other end of the spectrum, an alcohol dependent subject in later stages of alcoholism typically has a life that is quite unmanageable, often denies that he or she has a problem, and often drinks more than intended in an attempt to suppress feelings such as anger, depression and social discomfort. Cessation of heavy alcohol use, once a medical risk associated with a non-negligible mortality, has now long been possible to achieve using pharmacotherapy (Mayo-Smith 1997). While much of resources continue to be devoted to short term withdrawal management, it is instead prevention of relapse that poses a challenge. In the absence of effective pharmacotherapies, 60–70% of “detoxified” alcoholics relapse within the first year (Hunt et al. 1971). For a recovering alcohol abstinent subject, situations where there is alcohol or alcohol-related paraphernalia, every-day stressors, or sampling a small amount of alcohol (“a slip”) are potent relapse triggers (Larimer et al. 1999), much like drug associated cues, stress or priming doses of drug are able to reinstate drug-seeking in experimental animals (Shaham et al. 2003). For most, although perhaps not all recovering alcoholics, complete abstinence from alcohol is therefore critical to prevent a return to heavy, uncontrolled drinking (Sobell and Sobell 1993). Developing pharmacotherapies that will enable these subjects to control their behavior in situations that would otherwise drive them to drink will therefore address a key therapeutic need.

3.1 Neuronal Nicotinic Receptors

Extensive preclinical evidence supports the view that hedonic and addictive properties of natural rewards and substances of abuse are in part mediated by the mesolimbic/cortical DA system (Koob 1992; Schultz 2002; Wise and

Rompre 1989), including rewarding effects of nicotine (Di Chiara 2000) and ethanol (Brodie et al. 1990; Gessa et al. 1985; Brodie et al. 1999; Brodie 2002; Bunney et al. 2001). Increasing evidence suggests that neuroadaptations within the mesolimbic/cortical system are involved in the initiation and expression of addiction (Robinson and Berridge 2000; Wolf 1998; Kalivas and Stewart 1991; Vanderschuren and Kalivas 2000). A seminal human study close to 40 years ago suggested a key role for DA in pleasurable and stimulating effects of alcohol (Ahlenius et al. 1973), and numerous microdialysis studies in rodents subsequently demonstrated an ability of alcohol to release DA in the nucleus accumbens (NAc), although to a lower degree than other addictive drugs (Di Chiara and Imperato 1988; Imperato and Di Chiara 1986). In humans, alcohol has been shown to activate the ventral striatum of non-dependent social drinkers, as measured by functional magnetic resonance tomography (fMRI) (Gilman et al. 2008), and positron emission tomography (PET) using the D2 receptor ligand ^{11}C-raclopride has shown a modest DA release in response to an oral alcohol challenge in social drinkers (Boileau et al. 2003).

In this context, a key observation is that acute administration of either nicotine or ethanol increases DA levels in the NAc (Blomqvist et al. 1997). Ethanol can modulate the mesolimbic dopaminergic system via an increase in endogenous acetylcholine (ACh) and an interaction with neuronal nicotinic acetylcholine receptors (nAChRs) (Imperato et al. 1986; Blomqvist et al. 1997; Brodie 2002; Larsson et al. 2005; Blomqvist et al. 1993; Clarke et al. 1988). In fact, the most widely studied role of nAChRs is in the modulation of neurotransmitter release such as dopamine (Ericson et al. 2009; Tizabi et al. 2007). The isolation of the nAChR followed several decades of research and discovery leading to the characterization of nAChRs as pentameric ligand-gated ion channels consisting of different combinations of α2–α10 and β2–β4 subunits (for review: (Albuquerque et al. 2009; Champtiaux et al. 2003; Colquhoun and Patrick 1997; Gotti et al. 2009; Luetje et al. 1990; Patrick et al. 1993; Sargent 1993)). The importance of nAChRs in the human brain is now well established and appreciated as indicated by several excellent reviews on their molecular and functional structure (Gotti et al. 2009; Deneris et al. 1991; Lindstrom et al. 1990), anatomical distribution (Le Novere et al. 2002; Gotti et al. 2006), physiology (McGehee and Role 1995) as well as therapeutic indication for the treatment of several diseases such as Alzheimer's disease, schizophrenia, epilepsy, pain, Parkinson's disease and nicotine and alcohol dependence (D'Hoedt and Bertrand 2009; Dani and Bertrand 2007; Vazquez-Palacios and Bonilla-Jaime 2004; Gotti and Clementi 2004).

In the mammalian brain, most of the nAChRs contain both the α4* and β2* subunit that form heteromeric receptors and the homomeric α7 subunit-containing receptors (Flores et al. 1992; Corringer et al. 2000; Zoli et al. 1998; Le Novere and Changeux 1995; Pabreza et al. 1991; Clarke et al. 1985). The asterisks (example: α4*) here onward indicate that these subunits combine with other subunits to form functional receptors. In the peripheral nervous system, the predominant nAChR receptor subtype contains α7 and α3* subunits co-assembled with β2* or β4* subunits (Gotti et al. 1997). Although specific regions of the brain are often

predominated with single classes of nAChRs, the formation of other subclasses can also occur.

The mesolimbic dopamine pathway consisting of the ventral tegmental area (VTA), and the nucleus accumbens (NAc) has receptors mostly containing the $\alpha4^*$ and $\beta2^*$ subunits with or without the co-assembly with $\alpha6^*$ or $\alpha5^*$ subunits (Klink et al. 2001; Zoli et al. 2002). It is well established that the $\alpha4\beta2^*$ nAChRs have an essential role in mediating the reinforcing properties of nicotine (Crawley et al. 1997; Tapper et al. 2004; Picciotto et al. 1997). However, the subunit composition of the nAChR involved in the reinforcing effects of ethanol remains controversial.

3.1.1 The Role of nAChRs in Ethanol Self-Administration and Consumption

In vitro studies have shown that ethanol can directly activate $\alpha4\beta2$ nAChRs when heterologously expressed in Xenopus oocytes (Cardoso et al. 1999). Furthermore, the $\alpha4$ nAChR gene may influence some of the common actions of nicotine and ethanol. A polymorphism in the gene encoding the $\alpha4$ subunit of the nAChR (CHRNA4) is associated with altered ethanol sensitivity (Tritto et al. 2001), modulates ethanol withdrawal (Butt et al. 2004) and ethanol's effect on acoustic startle response (Tritto et al. 2001; Owens et al. 2003). It has been shown that administration of the non-specific nAChR antagonist, mecamylamine decreases ethanol intake (Blomqvist et al. 1996; Le et al. 2000a) and attenuates the stimulant and euphoric effects of alcohol (Chi and de Wit 2003). This indicates that nAChRs play a role in modulating ethanol consumption. Compared with other $\alpha4\beta2$ nAChR inhibitors, varenicline has high affinity and selectivity at $\alpha4\beta2$ nAChRs at the concentrations they reach in the brain (30 nM) (Coe et al. 2005a; Rollema et al. 2007). Varenicline has been approved for marketing in the USA (as Chantix^{TM}) and in over 30 countries worldwide (as Champix^{TM}) as an aid for smoking cessation (Gonzales et al. 2006; Tonstad et al. 2006; Jorenby et al. 2006). Varenicline reduces operant ethanol self-administration and heavy-drinking in rats following long-term ethanol exposure (Steensland et al. 2007). This supports studies from other investigators showing that dihydro-beta-erythroidine (DHβE), a selective $\alpha4\beta2$ nAChR antagonist, did not decrease ethanol intake using a limited access 6–12% two-bottle choice drinking paradigm (Larsson et al. 2005; Le et al. 2000a). This suggests that long-term exposure to ethanol induces specific changes in $\alpha4\beta2$ nAChRs. For example, an up-regulation of $\alpha4\beta2$ nAChRs in GABAergic neurons in the VTA has been found following chronic nicotine administration (Nashmi et al. 2007). Furthermore, it has been shown that long-term ethanol exposure (20 weeks) significantly increases the number of nAChR binding sites in the rat brain (Yoshida et al. 1982; Booker and Collins 1997). Together, this suggests that both the length of time and the amount of ethanol consumed induces changes in the function of nAChRs.

3.1.2 The Role of nAChRs in the VTA Following Short and Long-Term Ethanol Consumption

While nAChRs are expressed throughout the CNS, nicotine increases DA efflux in the NAc by directly stimulating nAChRs present in the VTA (Nisell et al. 1994; Maskos et al. 2005). Similarly, ethanol has been shown to both directly and indirectly modulate the mesolimbic DA reward circuitry by increasing ACh efflux in the VTA, an effect that is inhibited by mecamylamine, the non-specific nAChR antagonist (Larsson et al. 2005). The administration of concentrations of ethanol into the NAc comparable to those that would be expected following moderate intoxication have no effect on extracellular DA levels (Yim et al. 1998; Budygin et al. 2001). This suggests that ethanol facilitates DA release in the NAc by increasing the firing rate of dopaminergic neurons in the VTA (Brodie et al. 1990; Bunney et al. 2001; Brodie 2002; Brodie et al. 1999; Gessa et al. 1985). Most nAChR subunits ($\alpha3$–$\alpha7$ and $\beta2$–$\beta4$) are expressed in the VTA and there is evidence for functional nAChRs on dopaminergic and GABAergic cell bodies (Klink et al. 2001). It has been proposed that $(\alpha4)_2$ $(\beta2)_3$ nAChRs reside on GABAergic cell bodies in the VTA, whereas dopaminergic cells possess multiple hetero-oligomeric nAChRs with more complex subunit compositions (Champtiaux et al. 2003; Cui et al. 2003).

Understanding the contribution of specific hetero-oligomeric nAChRs in ethanol-mediated behaviors is difficult as the pharmacological tools available are limited, therefore the precise role of individual nAChRs in the effects of ethanol is still not known. It has been shown that ethanol-induced DA release in the NAc is reduced by the non-selective nAChR antagonist, mecamylamine, administered either systemically or intra-VTA but not intra-NAc (Blomqvist et al. 1997; Tizabi et al. 2002; Ericson et al. 1998). This suggests that nAChRs in the VTA modulate DA efflux in the NAc. Furthermore, it has been shown there is an increase of extracellular acetylcholine (ACh) levels in the VTA in rats that are voluntarily consuming ethanol ($\sim$0.7 g/kg/h), and an almost time-locked increase of DA levels in the NAc (Larsson et al. 2005). Also, an acute systemic dose of alcohol combined with a central injection of nicotine into the VTA result in an additive release of DA in the shell of the NAc (Tizabi et al. 2002). The selective $\alpha4\beta2$ nAChR antagonist, DHβE, administered into the VTA did not reduce DA efflux in the NAc induced following acute ethanol administration in rats, measured using in vivo microdialysis (Larsson et al. 2002; Ericson et al. 2003; Larsson et al. 2004). However, in mice, administration of the $\alpha3\beta2$ and $\alpha6\beta2$ nAChR antagonist, α-conotoxin MII, into the VTA significantly reduced ethanol-induced DA efflux in the NAc. This suggests that $\alpha3\beta2$ and $\alpha6\beta2$ nAChRs play an important role in the acute effects of ethanol. Together, these results indicate that nAChRs in the VTA play a significant role in modulating mesolimbic dopaminergic circuits. In addition to dopaminergic neurons, GABAergic neurons appear to be critical regulators of mesocorticolimbic DA neurotransmission, as GABAergic neurons in the VTA are sensitive to local and systemic effects of ethanol. Acute administration of ethanol reduced the firing rate of VTA GABAergic neurons and chronic ethanol administration enhanced the baseline activity of VTA GABA neurons and induced

tolerance to ethanol inhibition of their firing rate (Gallegos et al. 1999). This means that chronic ethanol administration may up-regulate VTA GABA neuronal activity. It has been hypothesized that the increase in VTA GABA neuron excitability underlies the decrease in mesolimbic dopamine neuronal activity and release of dopamine associated with withdrawal from chronic ethanol (Diana et al. 2003). Both the non-selective nAChR antagonist mecamylamine and varenicline, a partial agonist at $\alpha4\beta2$* nAChRs, reduce the rewarding properties of alcohol in ethanol-preferring rats (Blomqvist et al. 1997; Steensland et al. 2007; Ericson et al. 1998; Le et al. 2000a; Blomqvist et al. 1996) and in humans (Young et al. 2005; McKee et al. 2009; Blomqvist et al. 2002). These studies suggest that nAChRs are important therapeutic targets for the treatment of alcohol use disorders. While, several studies have established that the $\alpha4\beta2$* nAChR has an essential role in mediating nicotine's rewarding properties (Crawley et al. 1997; Tapper et al. 2004; Picciotto et al. 1997), the role of $\alpha4\beta2$* nAChRs in ethanol-mediated behaviors is more controversial (Tritto et al. 2001; Steensland et al. 2007; Butt et al. 2005; Owens et al. 2003; Larsson et al. 2002; Le et al. 2000a), as it has been shown that alpha-conotoxin MII, an antagonist at $\alpha3\beta2$*, $\beta3$* and/or $\alpha6$* nAChRs reduced ethanol intake (Larsson et al. 2004) and operant ethanol self-administration (Kuzmin et al. 2009).

Over the past few years, human genetic association studies have implicated a genetic locus, which includes the CHRNA5-CHRNA3-CHRNB4 gene cluster, encoding $\alpha5$, $\alpha3$ and $\beta4$ nAChR, in alcohol and nicotine consumption and dependence (Bierut et al. 2008; Wang et al. 2009; Saccone et al. 2009; Chen et al. 2009; Stevens et al. 2008; Joslyn et al. 2008; Grucza et al. 2008). However, there have been few ligands available to evaluate the role of $\alpha3$* or $\beta4$* or $\alpha5$* nAChRs in ethanol-mediated behaviors, this is currently being investigated.

3.1.3 The Role of nAChRs in the Reinstatement of Ethanol Seeking

In addition to drug-induced reinstatement, stress and exposure to situations previously associated with reward seeking also significantly contribute to relapse. In previous studies, it has been shown that stressors induce reinstatement of ethanol seeking (Liu and Weiss 2002). A common stressor for inducing reinstatement of ethanol seeking in rats is intermittent footshock (Liu and Weiss 2002; Le et al. 1999; Le et al. 1998; Liu and Weiss 2003), and recently a pharmacological stressor, yohimbine, has proven to induce reinstatement of ethanol, palatable-food and methamphetamine seeking in rats (Ghitza et al. 2006; Richards et al. 2008; Shepard et al. 2004; Le et al. 2005). In animal studies, it has been reported that nicotine is a potent stimulus for the secretion of stress-responsive hormones, such as corticosterone (Benwell and Balfour 1979; Cam and Bassett 1984) and induces ACTH secretion from the anterior pituitary by directly activating noradrenergic areas of the nucleus tractus solitarius (Matta et al. 1987; Zhao et al. 2007). This suggests that nAChRs are activated by exposure to stress. It has been previously shown that nicotine, a full agonist at $\alpha4\beta2$ nAChRs will reinstate ethanol seeking (Le et al. 2003), suggesting that $\alpha4\beta2$ nAChRs play a role in stress-induced reinstatement.

3.1.4 The Role of nAChRs in the Amygdala in Cue and/or Stress-Induced Reinstatement of Drug-Seeking

Compulsive drug-seeking and relapse may be driven by several factors that involve stress or anxiety (for review see (Heilig and Koob 2007). The stress-related structures in the brain, particularly the extended amygdala, have been implicated in multiple aspects of addiction. The extended amygdala consists of the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and shell of the nucleus accumbens (NAc-Sh) (Alheid et al. 1998). This system is important in several stress-related components of drug withdrawal (Smith and Aston-Jones 2008). In animal models of stress-induced reinstatement of drug-seeking, animals are trained to self-administer a drug across several sessions and then given non-reinforced trials to extinguish the drug-seeking response. Intermittent footshock delivered in the operant self-administration chamber potently reinstates lever-pressing in the absence of drug reward; this is considered a measure of drug-seeking (Le et al. 1998; Liu and Weiss 2003). The work of several laboratories has shown that such reinstatement of drug-seeking is dependent upon the extended amygdala. For example, inactivation of CeA, BNST, NAc-Sh or VTA blocks footshock-induced reinstatement of cocaine seeking (McFarland et al. 2004). Several observations suggest that nAChRs may be localized to the presynaptic terminals in the amygdala and nAChR binding studies have revealed nAChR protein in the amygdala (Hill et al. 1993; Hunt and Schmidt 1978). Hence cholinergic afferents to the amygdala may release endogenous acetylcholine and may modulate transmission in the amygdala via presynaptic nAChRs. Considerably less is known regarding the effects of nicotine in the CeA, although a recent study suggested that nicotine may enhance glutamate-mediated transmission in the mouse amygdala (Barazangi and Role 2001). Furthermore, chronic exposure to nicotine promotes the induction of long-lasting modifications of synapses in a specific pathway in the amygdala that is prevented by blocking nAChRs (Huang et al. 2008).

There is a high probability that a heavy drinker may also be a heavy smoker, suggesting that nAChRs are potential therapeutic targets for the treatment of alcohol use disorders (AUDs) and that the use of FDA-approved nAChR medications, such as varenicline and mecamylamine, approved as smoking cessation aids may prove to be valuable treatments for AUDs.

3.1.5 Translating Basic Research on Nicotinic Receptors into the Clinic

Mecamylamine

Mecamylamine is a non-specific nicotinic receptor antagonist and was first made available as an oral hypertensive drug in the early 1950s. Preclinical studies have shown mecamylamine reduces the rewarding properties of nicotine effectively (Rose et al. 1994). A few human studies have evaluated the effect of

mecamylamine on the subjective response of healthy individuals to moderate intake of alcohol. In a small scale study of ten men and woman healthy volunteers with no history of other substance use disorders were given alcohol–containing drinks and mecamylamine reduced the breath alcohol levels in comparison to placebo during the ascending limb of the blood alcohol levels (Blomqvist et al. 2002). Mecamylamine also reduced the drug effect questionnaire and alcohol sensation scale, thereby showing the potential to reduce both the pharmacokinetic and the rewarding profile of alcohol in human subjects.

Varenicline

At the beginning of this decade, the only approved therapies available for tobacco dependence were nicotine replacement with multiple delivery methods and the antidepressant bupropion. The discovery of varenicline by Pfizer Inc as a smoking cessation was based on the need to find improved long-term efficacious treatment (Coe et al. 2005b). Varenicline was developed with partial agonist activity (45% vs. nicotine) and strong binding affinity (0.06 nM) at $\alpha 4\beta 2^*$ nAChRs. It was hypothesized that an effective agent would be one that exhibits intrinsic partial agonist activity at $\alpha 4\beta 2^*$ nAChR, eliciting a moderate sustained release of mesolimbic dopamine. It was anticipated that the intrinsic partial agonist activity would then counteract the low dopamine levels experienced in the absence of nicotine during smoking cessation attempts which mediate craving and eventually relapse to smoking behavior. The high-affinity partial agonist would have the advantage of blunting, craving and withdrawing, as well as blocking nicotine-induced dopamine activation of $\alpha 4\beta 2^*$ nAChRs by competitively binding to this receptor subtype. The blocking of the nicotine-induced elevation of dopamine would thereby prevent the reinforcing and rewarding properties of tobacco. Soon after varenicline was marketed as a smoking cessation aid, preclinical (Steensland et al. 2007) and subsequently clinical studies (McKee et al. 2009) investigating the pharmacotherapeutic potential of this novel drug for the treatment of AUDs were initiated. The first small scale human study of 20 heavy-drinking smokers was conducted by Dr. McKee and colleagues in a double-blind, placebo-controlled investigation to test the medication effects of varenicline on the reactivity to a priming drink and subsequent alcohol self-administration behavior (McKee et al. 2009). After 7 days of pretreatment with varenicline (2 mg/day) or placebo, a priming dose (0.3 g/kg) of alcohol was administered for which the subjective and physiologic responses were assessed. This was followed with a 2-hour self-administration period during which the subjects could choose to consume up to eight additional drinks (0.15 g/kg). In this study, varenicline significantly reduced the number of drinks compared to placebo and increased the likelihood of remaining abstinent during the 2 hour self-administrating period. Varenicline also attenuated alcohol craving and subjective reinforcing alcohol effects following the consumption of the priming alcohol drink. The authors suggested that since the effect of varenicline on drinks consumed and craving responses were similar to the

effects of naltrexone using an identical laboratory paradigm (O'Malley et al. 2002), the observed effects, albeit in a small sample, had clinical relevance.

In this study, varenicline was well-tolerated in heavy-drinking smokers and the side effects experienced during the pretreatment were minimal and did not differ between the varenicline and placebo treatment groups. During the self-administration session, varenicline in combination with alcohol showed no visible effect on mood ratings, physiologic reactivity or adverse effects such as nausea, jitteriness or dizziness.

Therefore, varenicline administered with low doses of alcohol appears safe and well-tolerated in heavy-drinking smokers. Although this study was conducted in heavy-drinking smokers, they were not deprived of cigarettes and hence varenicline's effect was independent of nicotine withdrawal. Future studies with larger sample size of heavy drinkers who are smokers and non-smokers need to be conducted for the development of this compound for AUDs.

To address the growing concerns of the risk of suicidal behavior associated with varenicline, a very large scale study ($n = 80{,}660$) of men and women between the ages 18 and 95 was recently conducted (Gunnell et al. 2009). The investigators measured outcomes such as fatal and non-fatal self-harm, suicidal thoughts and depression following varenicline prescription and alternative smoking cessation treatments such as bupropion and nicotine replacement products. In this study, the authors found no clear evidence that varenicline treatment produced an increased risk of self-harm, depression or suicidal thoughts, compared with the alternative smoking cessation treatments. Additionally, others studies have shown that individuals including some mentally-ill subjects have benefited from taking varenicline with negligible side effects, with no worsening of neuropsychiatric symptoms or mood disturbances (Stapleton et al. 2008; McClure et al. 2009; Ramon and Bruguera 2009; Grosshans et al. 2009; Philip et al. 2009; Ochoa 2009; Smith et al. 2009). Therefore, it may be that varenicline is effective as a smoking cessation aid for some individuals and not for others, and patients prescribed this drug need to be monitored carefully for behavioral changes to prevent any serious psychiatric outcomes. It is worth noting that for the treatment of AUDs, the effect of varenicline appears to be effective, safe and well-tolerated in heavy-drinking smokers, albeit in small scale study, however, future large scale study is imperative.

3.2 Corticotropin-Releasing Hormone

An important theme that has emerged from recent research is that progression from social alcohol consumption into alcoholism is characterized by extensive long-term changes, or neuroadaptations, in brain systems that control behavioral stress responses and negative affects. Studies of these long-term neuroadaptations have long been limited by methodological difficulties. In contrast to for example cocaine or heroin, sufficient levels of voluntary alcohol consumption to induce dependence cannot be easily achieved in most species of experimental animals.

A practical solution to this dilemma was offered by the use of alcohol vapor inhalation, a model first established in the seventies (Goldstein and Pal 1971). Vapor inhalation allows precise control of brain alcohol exposure and makes it possible to emulate a level, pattern and duration of exposure that shares key characteristics with what occurs in clinical alcoholism. Using this approach, it has been shown that prolonged brain alcohol exposure at intoxicating levels leads to persistent behavioral consequences that seem to be relevant for alcoholism (Roberts et al. 2000; Rimondini et al. 2002). A prolonged duration (Rimondini et al. 2003) and an intermittent pattern of exposure (Rimondini et al. 2002; O'Dell et al. 2004), two features that mimic the exposure profile in clinical alcoholism, appear critical for induction of the behavioral changes. Other methods that lead to repeated cycles of intoxication and withdrawal exist, such as through forced liquid diet. Although perhaps less potent and less easy to control, these appear to induce a similar set of behavioral consequences [for review, see (Breese et al. 2005a)].

Prolonged brain alcohol exposure in experimental animals results in two key behavioral consequences: escalation, or a progressive increase of subsequent voluntary alcohol intake, measured both using simple two-bottle free-choice drinking (Rimondini et al. 2002; Griffin et al. 2009; Lopez et al. 2008; Lopez and Becker 2005) and operant responding for alcohol (Roberts et al. 2000); and sensitization of behavioral stress responses (Overstreet et al. 2002; Breese et al. 2005b; Valdez et al. 2004; Valdez et al. 2003; Valdez et al. 2002; Sommer et al. 2008).

A landmark paper described escalation as the escalation of drug self-administration that occurs with extended drug access (Ahmed and Koob 1998). In what may be an important difference between alcohol and other addictive drugs, experimenter-imposed brain alcohol exposure is sufficient to induce a similar progressive increase in voluntary drug intake? Following a prolonged history of dependence in this model, both escalation and behavioral sensitization to stress emerge during withdrawal, but persist long after withdrawal symptoms have resolved, and may in fact be very long-lasting. The term "post-dependent" has been introduced to reflect the sum of neuroadaptations that are induced as an individual becomes dependent on alcohol, and that remain for extended periods of time thereafter. These neuroadaptations can be maintained by continued brain alcohol exposure, but one of their key characteristics is that they remain even in its absence. Although not studied in detail until now, the extent and duration of post-dependent neuroadaptations show considerable individual variability. It can be hypothesized that, based on genetic susceptibility and other factors, the neuro-adapted, post-dependent state remains indefinitely in some individuals ("once an alcoholic, always an alcoholic"), while in others it remits.

Among the two behavioral characteristics of the post-dependent state, escalation of intake obviously mirrors a core characteristic of clinical alcoholism. The other, persistent sensitization to stress, has now consistently been shown using a range of models, including the elevated plus-maze (Valdez et al. 2003), social interaction (Overstreet et al. 2002) and fear-suppressed (conflict) responding in rats (Sommer et al. 2008). These observations are interesting given that anxiety

symptoms seem to be present regardless of alcoholism subtype (Ducci et al. 2007), but are highly contentious in clinical alcoholism research [see e.g. (Schuckit and Hesselbrock 1994)]. The dynamic nature of dependence-induced sensitization to stress is probably key to resolving this issue. The following set of observations provide perhaps the clearest demonstration of this pattern (Valdez et al. 2003). Seven weeks after completion of alcohol exposure, a history of dependence did not seem to result in any anxiogenic effect on the elevated plus-maze under unchallenged conditions. This is in agreement with clinical observations, in which established clinical ratings of anxiety in most patients decline over 3–6 weeks to clinically insignificant levels (Schuckit and Hesselbrock 1994). A very different picture emerged, however, when plus-maze testing was preceded by a restraint stress. Importantly, the magnitude of the stressor was chosen so that it did not result in an anxiogenic effect in animals without a history of alcohol exposure. In animals with a prolonged history of alcohol dependence, however, a potent anxiogenic effect was observed in this case. In a conflict model, where the anxiety testing itself is carried out under stressful conditions, the sensitized response of animals with a history of dependence is observed without additional manipulations (Sommer et al. 2008).

Some human translation of these findings are already available. Detoxified alcoholics have up-regulated brain responses to negative affective stimuli from the International Affective Picture System [IAPS; (Lang et al. 1995)], as measured by fMRI (Gilman and Hommer 2008). Interestingly, a key component of the network where sensitized activation is seen is the insula, a brain region that encodes negative interoceptive states, and whose anterior part has a high degree of reciprocal connectivity with the amygdala complex (Naqvi and Bechara 2009). Insula activation correlates with subjective measures of craving (Brody et al. 2002), and loss of this structure caused a remarkable disruption of cigarette smoking (Naqvi et al. 2007). The sensitized brain responses to negative IAPS pictures were observed a minimum of 3 weeks into abstinence, a time point at which conventional anxiety ratings are typically back to clinically insignificant levels. Thus, taking together the animal and the human observations, the post-dependent state may not necessarily be characterized by an increased anxiety, but rather by up-regulated reactivity to stressors. Given the critical role of stressors to trigger relapse (Brownell et al. 1986; Shaham et al. 2003), this is likely of importance for relapse vulnerability.

A history of dependence also appears to link sensitization of stress responses to escalation of voluntary alcohol intake. A common perception holds that stress generally increases alcohol intake, but this phenomenon is in fact not typically observed in non-dependent animals [see e.g. (Vengeliene et al. 2003)]. The picture, however, becomes very different following a history of dependence. As indicated above, even under non-stressful conditions, these animals start out with a higher level of voluntary alcohol consumption. Following exposure to stress, they escalate their intake further, and maintain it at this higher level even after the stress exposure has been terminated (Sommer et al. 2008). It would therefore appear from these observations that long-term neuroadaptations in alcohol dependence

not only lead to escalation of alcohol intake and sensitization of stress responses, but also create a connection between these two behaviors.

Recruitment of extrahypothalamic corticotrophin-releasing hormone (CRH) transmission is a key neuroadaptive mechanism underlying the behavioral traits described above. CRH is best known as the hypothalamic release hormone for ACTH (Vale et al. 1981), but extensive networks of CRH expressing neurons are also present in extrahypothalamic structures, including the central nucleus of the amygdala (CeA) and bed nucleus of stria terminalis (BNST), two components of the extended amygdale that are critical for stress responses and emotionality (Swanson et al. 1983). Behavioral stress responses are largely mediated by extrahypothamic CRH_1 receptors, primarily in the amygdala and BNST. Effects of CRH_2 activation are more variable and region dependent, but are frequently opposite to those of CRH_1 (Muller and Wurst 2004; Makino et al. 2002).

A critical feature of CRH signaling in behavioral stress responses may help understand the dynamic, activity dependent nature of sensitized stress responses in later stages of alcoholism. Neuropeptides are commonly released only at high neuronal firing frequencies, making them "alarm systems" that are not engaged under physiological or near-physiological conditions (Hokfelt et al. 1984). In agreement with this principle, extrahypothalamic CRH signaling seems to be largely quiescent unless activated by exposure to uncontrollable stress (Griebel et al. 2002; Gully et al. 2002).

Evidence has long been available to show that CRH activity within the amygdala and/or BNST drives acute alcohol withdrawal anxiety (Merlo et al. 1995; Olive et al. 2002; Baldwin et al. 1991; Rassnick et al. 1993; Gehlert et al. 2007). Of greater importance for the chronically addicted state, recent advances have shown that the persistent sensitization of behavioral stress responses following a history of dependence, described above, is also driven by CRH activity (Valdez et al. 2003; Funk et al. 2007; Funk et al. 2006a; Sommer et al. 2008). Similar results have been obtained using other means to induce alcohol dependence and the associated long-term neuroadaptations (Overstreet et al. 2002; Knapp et al. 2004; Overstreet et al. 2004; Breese et al. 2005b).

Similar to the sensitized stress responses, escalated self-administration or intake of alcohol following a history of dependence are also driven by up-regulated activity of extrahypothalamic CRH. Post-dependent animals tested 2 hours into withdrawal exhibit markedly elevated rates of self-administration, and these are brought down to non-dependent levels by systemic treatment with a whole range of CRH_1 selective antagonists. Showing the different nature of escalated versus baseline alcohol self-administration, none of the antagonists affected self-administration in non-dependent animals (Funk et al. 2007). In a follow-up study, the non-selective peptide CRH antagonist D-Phe $CRH_{12–41}$ microinjected into the CeA blocked excessive post-dependent self-administration rates, while microinjections into BNST or the Nc. Accumbens shell were ineffective. Furthermore, CeA injections of D-Phe $CRH_{12–41}$ in animals without a history of dependence were also ineffective, once again demonstrating that the CRH system, presumably

within the amygdala, is recruited to drive excessive alcohol self-administration in the post-dependent state (Funk et al. 2006a).

Suppression of escalated alcohol self-administration by CRH antagonism as outlined above was observed during acute withdrawal, but escalated alcohol self-administration has also been found long after forced alcohol exposure, and is equally sensitive to CRH or CRH_1 antagonism (Rimondini et al. 2002; Valdez et al. 2002; Gehlert et al. 2007). Intracerebroventricular administration of a CRH antagonist blocked escalated alcohol intake both during acute withdrawal and protracted abstinence, but did not affect basal alcohol intake in animals without a history of dependence (Valdez et al. 2002). Similarly, dependence induction using gastric gavage followed by cycles of self-administration and imposed deprivation periods, also resulted in excessive self-administration. After several weeks, the novel selective non-peptide CRH_1 antagonist MTIP suppressed alcohol self-administration in post-dependent animals to non-dependent levels, while the same doses of MTIP were inactive in animals without a history of dependence (Gehlert et al. 2007). In summary, a pathological engagement of extrahypothalamic CRH activity drives escalated alcohol intake in animals with a history of dependence, both during withdrawal and long after withdrawal has subsided.

As indicated above, stress is a major trigger for relapse in alcoholics, and reinstates previously extinguished alcohol-seeking in experimental animals (Brownell et al. 1986; Shaham et al. 2003). Both non-selective and CRH_1 selective CRH antagonists block stress-induced reinstatement, but do not influence reinstatement triggered by alcohol associated stimuli, which in contrast is blocked by naltrexone. The ability of CRH blockade to suppress stress-induced relapse-like behavior is mediated through extrahypothalamic CRH systems (Le et al. 2000b; Liu and Weiss 2002). Post-dependent animals display a markedly increased sensitivity to blockade of stress-induced reinstatement by CRH antagonism (Gehlert et al. 2007). The selective CRH_1 antagonist MTIP entirely blocked this behavior at 10 mg/kg, a dose at which no effect was seen in animals without a history of dependence. Taken together, these data show that CRH_1 receptors mediate stress-induced reinstatement, and that a recruitment of the CRH system in the post-dependent state renders animals preferentially sensitive to blockade of relapse-like behavior by CRH_1 antagonism.

The α_2-adrenergic antagonist yohimbine, a pharmacological stressor that can substitute for foot-shock to reinstate alcohol seeking (Le et al. 2005), has recently been shown to up-regulate CRH expression in CeA (Funk et al. 2006b). It is, however, unknown whether CRH within CeA fully or in part mediates stress-induced reinstatement, and in fact, CRH antagonist microinjections into the median raphe blocked relapse-like behavior in this model (Le et al. 2002). This suggests that multiple CRH pathways might be involved and act in concert to mediate different alcohol related behaviors.

Thus, recruitment of CRH signaling within the extended amygdala is a major factor behind increased stress sensitivity, excessive self-administration and relapse in the post-dependent state. The mechanisms through which this occurs are beginning to emerge. During acute alcohol withdrawal, release of CRH is

increased in the amygdala (Merlo et al. 1995). Presumably as a reflection of this, decreased tissue levels of CRH were seen within this structure in early withdrawal (Zorrilla et al. 2001; Funk et al. 2006a). Six weeks after last alcohol exposure, however, amygdala CRH had not only recovered, but also increased to supra-normal levels (Zorrilla et al. 2001). Elevated tissue content of CRH peptide in the amygdala could either reflect increased synthesis, or decreased utilization. Our finding of increased CRH transcript levels in the CeA during the post-dependent state supports increased synthesis in CeA in this condition (Sommer et al. 2008).

A major contribution to up-regulated CRH signaling, however, comes from an up-regulation of CRH_1 receptor expression and binding within the amygdala. This is consistent with the left-shifted dose–response curve for CRH_1 antagonists observed in animals with a history of dependence. Perhaps the best demonstration that CRH_1 up-regulation produces the characteristics of the post-dependent phenotype was obtained in the genetically selected, alcohol preferring Marchigian–Sardinian Preferring (msP) rat (Ciccocioppo et al. 2006). These animals essentially represent a behavioral phenocopy of post-dependent rats, with which they share increased stress reactivity, excessive self-administration of alcohol, and increased propensity for relapse-like behavior. A screen for differential gene expression in the msP-rat showed a marked up-regulation of the transcript encoding the CRH_1 receptor within the amygdala complex. This was linked to a *Crhr1* promoter variant unique to msP rats. In msP rats, the selective CRH_1 antagonist, antalarmin, reduced alcohol self-administration to levels found in genetically heterogeneous animals without a history of dependence. Antalarmin also blocked stress-induced reinstatement of alcohol-seeking in msP rats at doses that did not affect non-selected rats without a history of dependence (Hansson et al. 2006). This is a further parallel to the post-dependent phenotype. Interestingly, when msP animals were given *ad lib* access to alcohol, the ensuing consumption was sufficient to down-regulate the receptor transcript to normal levels (Hansson et al. 2007). Genetic variation at the *Crhr1* locus as a susceptibility factor for excessive alcohol drinking might have parallels in primates, including rhesus macaques (Barr et al. 2009) and humans, where a similar association was recently reported (Treutlein et al. 2006).

Following up on the msP findings, a similar up-regulation of CRH_1 expression was found in genetically non-selected, post-dependent rats (Sommer et al. 2008). This up-regulation persisted long after ethanol exposure, reflecting a long-term neuroadaptation rather than acute withdrawal. Similar to the msP findings, receptor up-regulation was most pronounced in the basolateral (BLA) and medial amygdala (MeA), and only to a lesser extent found in CeA (unpublished data). The relative contribution of specific amygdalar subnuclei to the post-dependent state remains to be established. Microinjections of a CRH antagonist in CeA blocked post-dependent excessive self-administration, but the BLA or MeA was not tested in that study (Funk et al. 2006a). Also, amygdalar nuclei are interconnected, and receptors expressed in BLA or MeA neurons could be inserted into terminals in other amygdala regions.

In summary, neuroadaptations that occur after a prolonged history of alcohol dependence seem to persist long after brain alcohol exposure and in some cases

perhaps for the lifetime of the individual. From a clinical perspective, a persistent vulnerability even after an extended period of sobriety has important implications for secondary prevention. Long-term neuroadaptations in alcoholism drive escalation of voluntary alcohol intake, behavioral sensitization to stress, and a concomitant sensitivity to stress-induced relapse. Up-regulation of CRH signaling within the amygdala complex appears to be a key mechanism behind these behavioral traits, and offers a promising target for new pharmacotherapies in alcoholism.

Clinical translation of preclinical findings validating CRH1 receptors as a target for alcoholism treatment has been slow. This experience illustrates better than most that target ID and validation is but one part of the process needed to bring a medication to patients. Despite early realization that targeting this mechanism might have a considerable potential in several stress-related clinical conditions, translation was held up for many years, because discovery of safe, orally available and brain penetrant molecules proved to be exceedingly difficult. The first molecule given to humans in an open label, uncontrolled depression trial, R121919 appeared to have some potential (Zobel et al. 2000), but was terminated from development because of a hepatic toxicity signal. Many early generation compounds were structurally related, with a similar potential for accumulation in the liver limiting their prospects for development. Once later generation compounds emerged, they were initially prioritized for development in anxiety and depression, where results have recently begun to emerge, and have not been encouraging (Binneman et al. 2008; Coric et al. 2010). It is our hypothesis that mood and anxiety disorders are highly heterogeneous, and the CRH system may not be consistently activated in these conditions. In contrast, as reviewed above, given sufficient duration of brain alcohol exposure, the CRH system does seem to be consistently and pathologically activated, offering the promise that results with CRH_1 antagonism in alcoholism will be more useful. Against this background, human translation of the preclinical reviewed above is now underway in one of our laboratories, using an experimental medicine approach described below.

3.3 Substance P and Its NK1 Receptor

Because CRH antagonists that would allow human translation were so slow in coming, we have explored whether other stress systems might act in parallel with CRH to drive excessive alcohol intake. We have been particularly interested in mechanisms that have been in clinical development for other indications, and therefore might offer molecules for which target engagement and acceptable safety in humans has already been demonstrated.

Substance P (SP) and its neurokinin 1 receptor (NK1R) appeared to be of interest in this context. SP is an 11 amino acid peptide originally isolated from intestinal extracts in 1931 (Euler and Gaddum 1931). For a long time, research on SP focused on nociceptive and inflammatory effects related to its role as a C-fiber

sensory transmitter [for review, see (Payan 1989)]. Hopes that blockers of SP transmission would become analgesic and anti-inflammatory treatments were, however, not fulfilled. Another category of possible indications was suggested by the fact that SP and its preferred NK1R are highly expressed in brain areas involved in stress responses, including the hypothalamus and the amygdala (Mantyh et al. 1984; Nakaya et al. 1994). Numerous observations also indicated a functional involvement of SP and NK1Rs in affective regulation. For instance, central injection of SP or related peptide agonists is anxiogenic in the elevated plus-maze (Teixeira et al. 1996) and causes conditioned place aversion (Elliott 1988). Conversely, NK1R antagonism or genetic deletion of the receptor is anxiolytic- and antidepressant-like in animal models (Teixeira et al. 1996; File 1997; Kramer et al. 1998; Santarelli et al. 2001; Varty et al. 2002; Papp et al. 2000; Ballard et al. 2001; Rupniak et al. 2000; Rupniak et al. 2001). Furthermore, activation of NK1R's resulting from release of endogenous SP has been linked to modulation of stress responses (Ebner et al. 2004; Ebner and Singewald 2007; Ebner et al. 2008b; Ebner et al. 2008a).

Based on these observations, NK1R antagonists have also been evaluated as possible therapeutics for affective disorders. However, despite initial findings in support of antidepressant and anxiolytic activity of NK1 antagonists in humans (Kramer et al. 1998; Kramer et al. 2004; Furmark et al. 2005), clinical efficacy for these indications has not been robustly replicated, and development has largely been discontinued. Similar to CRH, it is possible that NK1R signaling while important in some depressed subjects, may not be sufficiently uniformly involved in depression to produce a robust efficacy signal in heterogeneous patient populations. A more consistent activation of this system may be present in alcohol addiction, similar to the CRH findings reviewed above, and might render NK1R antagonism a more effective target mechanism for this indication. In addition, it has been reported that deletion of the *Tacr1* gene that encodes the NK1R blocks opiate reward (Murtra et al. 2000; Ripley et al. 2002; Gadd et al. 2003). Because endogenous opioids in part mediate alcohol reward, modulation of opioid mechanism by NK1R's could represent an additional mechanism through which NK1R antagonists contribute to altered alcohol reward.

In exploring these possibilities, we first established that genetic deletion of NK1R suppressed alcohol intake in a simple two-bottle free-choice drinking model. NK1R null mutant mice (De Felipe et al. 1998) were used after backcrossing into a C57BL/6 background for ten generations, so that a sufficient level of voluntary alcohol consumption would be present in control animals to allow detection of suppression by the receptor gene deletion (Crabbe and Phillips 2004). Wild-type (WT) control mice (NK1R +/+) ultimately consumed in excess of 10 g alcohol/kg/day at the end of a procedure in which alcohol concentration was gradually increased to 15% over 60 days. Alcohol consumption by NK1R −/− mice was markedly lower than that by WT controls. The difference was most prominent at higher alcohol concentrations, at which consumption motivated by pharmacological alcohol effects dominates over intake for non-pharmacological effects such as taste or calories (Crabbe and Phillips 2004). Relative preference for

alcohol was also markedly reduced, while water intake was unaffected by genotype (George et al. 2008).

Despite the concerns about cross-species activity mentioned above, we then found that in WT C57BL/6 mice, the NK1R antagonist L-703,606 suppresses alcohol intake in a manner that mimics the effects of genetically inactivating the NK1R. The antagonist was inactive in NK1R KO's, demonstrating that its effects to suppress alcohol drinking reflect activity at the target rather than off-target actions. Using a model recently developed by others (Melendez et al. 2006), we also found that escalation of alcohol intake after repeated cycles of deprivation was robustly detected in WT mice, but was absent in NK1R KO's. Finally, alcohol reward, as measured by conditioned place preference, is absent in NK1R KO's, perhaps indicating an involvement of this mechanism not only in negative but also in positive reinforcement by alcohol (Thorsell et al. 2010). These findings have since been shown using an operant self-administration model of ethanol reinforcement (Steensland et al. 2010).

Capitalizing on the availability of orally available, brain penetrant NK1R antagonists with demonstrated safety in humans, we have obtained a degree of human translation of the mouse NK1R findings. Using positron emission tomography (PET), we determined the relationship between dose and central NK1R occupancy (RO) for the high-affinity NK1R antagonist LY686017. Based on these data, we were able to select a dose, 50 mg daily, which yields a >90% blockade of central NK1R's. Human activity of the antagonist was evaluated in 50 recently detoxified anxious alcohol dependent subjects, hospitalized at the NIAAA inpatient unit throughout the duration of a 1 month experimental medicine trial. The predictive validity of individual surrogate markers is not well established, and a battery of experimental outcomes was therefore used. LY686017 produced a highly consistent profile of effects across the different outcomes. The antagonist suppressed spontaneous alcohol cravings, and had a beneficial effect on global measures of well-being, in the absence of effects on general psychopathology. Toward the end of the study, we exposed subjects to a challenge intended to simulate a situation under which relapse risk is high (George et al. 2008). To achieve this, we measured craving responses to a combination of an established stress challenge (TSST) (Kirschbaum et al. 1993), and equally established alcohol-cue exposure procedure (Monti et al. 1993). Treatment with LY686017 reduced both the subjective craving response to the combined challenge, and the concomitant cortisol response. Finally, we used fMRI to study the effects of LY686017 treatment on brain responses to standardized affective stimuli from the IAPS (Lang et al. 1995). As mentioned above, alcoholic's exhibit exaggerated behavioral and brain responses to images associated with negative affect, and conversely, exhibit reduced brain responses to standard positive images (Gilman and Hommer 2008). Brain responses in the placebo group were in agreement with those findings. In contrast, subjects treated with LY686017 had less activation to the negative images than the placebo group in several brain regions associated with emotional response to visual stimuli. In particular, the LY686017 group had less activation in the insula, as indicated above a brain region involved in craving

and addictive behavior (Naqvi and Bechara 2009). Unexpectedly, the LY686017-treated group also showed greater brain activation in the Nc. Accumbens and Anterior Cingulate Cortex to the positive IAPS images than the placebo treated group, essentially normalizing the deficit in brain responses to positive affective stimuli otherwise found in alcoholics. Together, the attenuation of responses to negative, and restoration of responses to positive affective stimuli may reflect an overall shift in the balance between positive and negative emotionality reflected in the subjective improvement detected by the clinical ratings.

In summary, NK1R antagonism has rapidly emerged as an attractive candidate treatment mechanism in alcoholism. It remains to be determined whether its beneficial effects are exclusively related to stress mechanisms, or whether effects on acute alcohol reward also contribute. More importantly, larger clinical trials that directly assess drinking outcomes under outpatient conditions are needed to determine the clinical potential of NK1R antagonists for treatment of alcoholism. Such trials are currently underway.

3.4 Orexin Receptors

Orexin-A (OX-A), also known as hypocretin, is a hypothalamic peptide that acts via orexin type 1 receptors (OX-R1). The most widely studied biological functions of orexins are the central control of feeding and sleep, however, in the past few years there have been findings that the orexin system modulates the hypothalamic–pituitary–adrenal (HPA) axis, acting on both its central and peripheral branches (for review see (Spinazzi et al. 2006). Orexin (OX) plays an important role in mediating behaviors such as motivational drive produced by drugs of abuse such as cocaine, morphine, nicotine and alcohol (DiLeone et al. 2003; Richards et al. 2008; Pasumarthi et al. 2006; Borgland et al. 2006; Paneda et al. 2005; Harris et al. 2005). In the central nervous system, OX-A is expressed in the lateral hypothalamus (LH) and orexin-containing neurons project throughout the brain, with a prominent input to basal forebrain structures involved in motivation, reward, and stress. In addition, neurons expressing these neuropeptides have extensive projections to regions of the brain important for behavioral responses to drugs of abuse, such as dopamine neurons of the VTA, raising the possibility that these pathways may also be important in addiction. Further, the extrahypothalamic distribution of OX-A parallels its involvement in affective behavioral responses to stress. For example, OX-induced reinstatement of cocaine-seeking was prevented by blockade of noradrenergic and corticotropin-releasing factor systems, suggesting that orexin-A reinstated drug-seeking through induction of a stress-like state (Boutrel et al. 2005). Extensive coexpression of tyrosine hydroxylase, a marker for dopamine neurons, with orexin receptors has been observed in the mouse VTA (Narita et al. 2006). An intra-VTA injection of a selective orexin receptor antagonist, SB334867, significantly suppressed morphine-induced place preference in rats (Narita et al. 2006). These findings provide new evidence that

orexin-A containing neurons overlap with VTA dopamine neurons and are implicated in behaviors associated with substance abuse.

Orexin has been shown to induce various behavioral changes related to adaptation to stress and has also been shown to play a key role in stress-induced reinstatement of cocaine seeking (Harris et al. 2005). An effective pharmacological treatment for alcohol use disorders would ideally prevent reinstatement of alcohol-seeking. Activation of lateral hypothalamic orexin neurons is strongly linked to preferences for cues associated with drug and food reward and is able to reinstate an extinguished drug-seeking behavior (Harris et al. 2005). OX-A induces potentiation of N-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission via a PLC/PKC-dependent insertion of NMDARs in VTA dopamine neuron synapses (Borgland et al. 2006). Furthermore, in vivo administration of an orexin-A receptor antagonist blocks locomotor sensitization to cocaine and occludes cocaine-induced potentiation of excitatory currents in VTA dopamine neurons (Borgland et al. 2006). These results provide in vitro and in vivo evidence for a critical role of orexin signaling in the VTA in neural plasticity relevant to addiction. This suggests that orexin in the VTA may play a significant role in stress-induced relapse to drug-seeking in drug-experienced animals. It has been shown that orexin-A/hypocretin-1 induces reinstatement and yohimbine-induced reinstatement can both be blocked by a CRF-1 receptor antagonist (Boutrel et al. 2005; Marinelli et al. 2007). In addition, yohimbine-induced increases in ethanol operant self-administration are inhibited by antalarmin, a CRF-1 receptor antagonist (Marinelli et al. 2007). Furthermore, yohimbine-induced reinstatement of palatable-food seeking is reduced by inhibiting CRF-1 receptors (Ghitza et al. 2006). Both yohimbine and CRF induce release of noradrenaline in the locus coeruleus (Chen et al. 1992). Yohimbine has also been shown to up-regulate CRF expression (Funk et al. 2006b) and there is evidence to suggest that the orexin/hypocretin system may augment arousal and evoke behavioral responses associated with fear, stress or emotion (Bisetti et al. 2006). There have been few human clinical studies to determine the validity of the animal studies primarily as there have not been any compounds available that target the orexin/hypocretin system for use in humans. However, companies are currently conducting Phase III clinical trials for sleep disorders; a “proof of concept” human study may be possible in the near term to investigate the role of these compounds in the treatment of alcohol use disorders.

4 Personalized Medicine and the Promise of Pharmacogenetics

Classical dopaminergic reward-related transmission is in part under γ-opioid receptor (OPRM1) control (Spanagel et al. 1992). Blockade of OPRM1 receptors in the VTA largely prevents alcohol-induced accumbal DA release, indicating that alcohol leads to release of endogenous opioids in the VTA (Tanda and Di Chiara 1998). It is therefore attractive to hypothesize that the opioid antagonist naltrexone

acts in part by disrupting this cascade. However, although meta-analyses of more than 30 randomized controlled trials with naltrexone support the efficacy of this treatment, the average effect size is modest (Bouza et al. 2004). One possible conclusion could be that endogenous opioid system activation by alcohol only plays a minor role in alcohol reward, excessive alcohol use, and alcoholism. If this is correct, then there would be no strong rationale for clinical use of treatments that target this mechanism. Unfortunately, this is the conclusion for most of the part has been drawn by clinicians, leading to a very limited clinical use of naltrexone (Mark et al. 2003). An alternative interpretation, that appears more appropriate in the age of personalized medicine, is that the limited overall effect size of naltrexone reflects heterogeneity of response among patients. There is particular reason to consider this possibility in the case of naltrexone, since the *OPRM1* locus encoding its target was discovered to harbor functional variation more than a decade ago (Bond et al. 1998). This A118G single nucleotide polymorphism (SNP) encodes an amino acid substitution in the N-terminal, extracellular loop of the receptor protein, at a putative glycosylation site. Clinical experience has long indicated marked heterogeneity of naltrexone responses. Research data have recently accumulated to suggest that the *OPRM1* A118G SNP may account for a substantial proportion of this heterogeneity.

Secondary analyses of clinical trials suggest that family history of alcoholism predicts clinical naltrexone response (Rubio et al. 2005). Direct support for this notion is also found under laboratory conditions, both with regard to subjective alcohol effects (King et al. 1997) and alcohol self-administration (Krishnan-Sarin et al. 2007). Although a role of family history clearly could reflect either genetic or environment factors or both, emerging evidence strongly suggests a major role of pharmacogenetic factors.

Clinical evaluation of pharmacogenetic factors poses numerous challenges unless studies are specifically designed to detect them. Most fundamentally, unless subjects are a priori recruited based on genotype, there is always a bias against detecting effects confined to carriers of a minor allele. Studies in rodent models are of limited utility to address this type of question because genetic variants that are functionally equivalent to those found in humans are rarely if ever found in species that are phylogenetically so distant. In contrast, functional equivalents of behaviorally important human variants have frequently arisen also in non-human primates (Barr and Goldman 2006). This is of evolutionary interest in its own right, but additionally offers a critical resource for addressing questions of addiction vulnerability and pharmacogenetics.

Accordingly, an *OPRM1* SNP that is functionally equivalent to the human A118G polymorphism (C77G) has been identified in rhesus macaques (Miller et al. 2004). Using this model, we found increased psychomotor stimulation in response to alcohol, increased alcohol preference, and increased frequency of alcohol consumption at a level leading to intoxication in carriers of the rhesus (rh) *OPRM1* 77G variant (Barr et al. 2007). Because psychomotor stimulation is a proxy marker of mesolimbic DA activity, these findings suggested that activation of classical brain reward systems in response to alcohol primarily or perhaps even

exclusively occurs in carriers of the rhesus 77G variant. A testable hypothesis prompted by these findings was that 77G carriers should be preferentially sensitive to suppression of alcohol preference by naltrexone. We used a short term treatment model and social drinking in non-dependent rhesus macaques to evaluate this hypothesis. In agreement with our prediction, naltrexone only suppressed alcohol preference in carriers of the rhesus 77G variant (Barr et al. 2009). Both the rhesus and the human data may have their own limitations, but they are highly complementary. Together, the picture that emerged is consistent with that suggested by the human secondary analyses that support a role of 118G as a predictor of treatment efficacy (Oslin et al. 2003; Anton et al. 2008).

The non-human primate and human data are also complementary in another aspect, in that they allow isolating the influence of C77G (in rhesus) and A118G (in humans) from that of other functional polymorphisms with which the respective variants might be in linkage disequilibrium (LD) in the two species. For instance, one human study found that other polymorphisms within the same haplotype block, but not A118G, were associated with diagnoses of substance dependence (Zhang et al. 2006). In contrast, a haplotype based re-analysis of the COMBINE study found naltrexone response to be specifically attributable to 118G (Oroszi et al. 2009). Furthermore, in humans, alternative isoforms of the μ-opioid receptor are encoded by transcripts that originate from different initiation sites, and genotype may therefore serve as a proxy for isoform identity (Shabalina et al. 2009). Combined, however, human and rhesus findings strongly suggest that the *rhOPRM1* C77G and the *hOPRM1* A118G SNPs, respectively, are functional with regard to alcohol as well as naltrexone response in the respective species.

Interestingly, our rhesus study in fact found opposite directionality of the naltrexone effect in 77G carriers and subjects homozygous for the major 77C allele. While alcohol preference was markedly suppressed in 77G carriers, there was a trend for increased preference in 77C homozygous individuals. This pattern parallels a human study that examined family history of alcoholism as a moderator of naltrexone response under laboratory conditions, and found suppression of self-administration in family history positive subjects, but significantly increased self-administration following naltrexone treatment in family history negative participants (Krishnan-Sarin et al. 2007).

Based on the work in non-human primates, we recently carried out a translational, or perhaps more appropriately "reverse-translational" study in humans and genetically modified mice. First, we directly determined alcohol-induced DA activity as a function of *OPRM1* A118G genotype in humans, using PET and ^{11}C-raclopride displacement. Alcohol-induced release of DA was studied in response to a pharmacokinetically controlled alcohol challenge in non-dependent, social drinkers, and evaluated whether it varies as a function of the human *OPRM1* A118G genotype. Throughout the striatum, displacement of the radioligand was only detected in 118G carriers, while the same measure in fact suggested reduced DA release in subjects homozygous for the major 118A allele following alcohol challenge. To isolate the influence of the A118G SNP from other variants in LD with it, we also generated two humanized mouse lines, in which the mouse *OPRM1* gene was replaced with the

human sequence. These mice carried a human *OPRM1* gene either with an A or a G in position 118, but were otherwise identical throughout their genome. Microdialysis of alcohol-induced DA release in the Nc. Accumbens showed a four-fold higher peak in the 118G compared to the 118A line (Ramchandani et al. 2010). Thus, presumably, it is only in 118G carriers that an activation of dopaminergic brain reward systems occurs in response to alcohol, and offers a mechanism for naltrexone to act on. Conversely, in the absence of such activation, administration of the antagonist would be expected to be largely silent. Besides demonstrating the critical role of the A118G SNP as a pharmacogenetic determinant, this set of studies illustrates that translation, taken seriously, is a two way street.

5 Final Comments

Basic neuroscience has identified numerous mechanisms that appear promising as targets for novel pharmacotherapies of alcoholism. The examples above, taken from the experience of our respective programs, are only a few among these. Clearly, other laboratories have pursued a large number of other mechanisms with equal degree of success. Together, we hope this illustrates that the field has made considerable progress since the days when many a paper started with statements such as “alcohol is a small, lipid soluble molecule, without specific sites of action in the central nervous system”. Furthermore, we have discussed the key challenges inherent in attempting to translate progress in the areas of target ID and validation into clinical treatments; the components that in our view need to be brought together to increase the chances of success in this challenging endeavor; and the characteristics of the teams, partnerships and environments where we believe chances for success can be maximized. In summary, clinical practice and epidemiology show that the unmet medical needs of alcohol dependent patients are enormous. Neuroscience indicates that the opportunities to address them is numerous, and that the time to do so is ripe. Perhaps the most important objective of this chapter is to share the urgency and the enthusiasm we personally feel in the course of attempting to do so, and the satisfaction we believe will come from even limited, incremental improvements in outcomes that will come with each addition to the treatment toolbox.

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New Pharmacological Treatment Strategies for Relapse Prevention

Rainer Spanagel and Valentina Vengeliene

Abstract Here we discuss treatment strategies that are based on pharmacological interventions to reduce craving and relapse in alcohol-dependent patients. We will first provide a historical overview about relapse prevention strategies. We will then review the development of disulfiram, naltrexone, acamprosate, and nalmefene and discuss their neurobiological modes of action. Then the concept of convergent genomic analysis will be introduced for the discovery of new molecular treatment targets. Finally, we will provide convincing evidence for the use of N-methyl-D-aspartate (NMDA) receptor channel blockers as substitution drugs. Important conclusions of this review are: (i) learning from other addictive substances is very helpful—e.g., substitution therapies as applied to opiate addiction for decades could also be translated to alcoholics, (ii) the glutamate theory of alcohol addiction provides a convincing framework for the use of NMDA receptor antagonists as substitution drugs for alcohol-dependent patients, (iii) a combination of behavioral and pharmacological therapies may be the optimal approach for future treatment strategies—one promising example concerns the pharmacological disruption of reconsolidation processes of alcohol cue memories, (iv) given that many neurotransmitter systems are affected by chronic alcohol consumption, numerous druggable targets have been identified; consequently, a "cocktail" of different compounds will further improve the treatment situation, (v) in silico psychopharmacology, such as drug repurposing will yield new medications, and

R. Spanagel (✉) · V. Vengeliene
Institute of Psychopharmacology, Central Institute of Mental Health,
Medical Faculty Mannheim, University of Heidelberg,
Square J5, 68159 Mannheim, Germany
e-mail: rainer.spanagel@zi-mannheim.de

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finally, (vi) the whole organism has to be taken into consideration to provide the best therapy for our patients. In summary, there is no other field in psychiatric research that has, in recent years, yielded so many novel, druggable targets and innovative treatment strategies than for alcohol addiction. However, it will still be several years before the majority of the "treatment-seeking population" will benefit from those developments.

Keywords Antabuse · Campral · Revia · Vivitrol · Nalmefene · Acetaldehyde dehydrogenase · Hyper-glutamatergic state · Kappa opioid receptors · Genome-wide analysis · Alcohol deprivation effect

Abbreviations

ADE	Alcohol deprivation effect
CRH	Corticotropin-releasing hormone
D3R	Dopamine D3 receptor
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance imaging
GHB	γ-Hydroxybutyric acid
GWAS	Genome-wide association study
GlyT	Glycine transporter
HPA	Hypothalamic–pituitary–adrenal
KOR	κ-Opioid receptor
MR	Magnetic resonance
MOR	μ-Opioid receptor
NMDA	N-metlyl-D-aspartat
Per	Period
RCT	Randomized controlled trial
RR	Relative risk
SNP	Single nucleotide polymorphisms
Ent1	Type 1 equilibrative nucleoside transporter

Contents

1 Introductory Remarks 585
2 Brief Historical Overview of Relapse Prevention Strategies 586
3 Current State of Pharmacological Relapse Prevention 589
4 Identification of New Drug Targets 591
5 Convergent Genomic Analysis for New Drug Target Definition 594
6 Treating a Hyper-Glutamatergic System 595
7 Concluding Remarks and Future Perspective 601
References 602

1 Introductory Remarks

This review focuses on treatment strategies that are based on pharmacological interventions to reduce craving and relapse in alcohol-dependent patients, although other non-pharmacological interventions may also be effective. For example, deep brain stimulation may become a treatment alternative especially for heavily dependent alcoholics. The first clinical studies employing bilateral deep brain stimulation in the nucleus accumbens in patients with severe and treatment-resistant alcohol abuse show that alcohol craving is greatly reduced and that the patients are able to abstain from drinking for extended periods of time (Kuhn et al. 2007, 2011; Müller et al. 2009; for more information see also "Deep brain stimulation as a therapy for alcohal addiction" from Thomas F. Münte). However, only a hand full of patients have so far undergone this treatment and much more research is needed, not only to see the effectiveness of this treatment, but also to understand its underlying mechanisms, identify its limitations, study its long-term consequences and side-effects, and most importantly, define its ethical implications. Psychotherapeutic-based interventions such as cue extinction training are promising as well, and it should be emphasized that each effective psychotherapeutic approach has a neurobiological underpinning. Hence, a recent randomized controlled trial (RCT) in abstinent alcohol-dependent patients using functional magnetic resonance imaging (fMRI) showed that extinction training impacts brain areas relevant for memory formation and attentional focus to alcohol-associated cues and affects the mesocorticolimbic reinforcement system (Vollstädt-Klein et al. 2011). In particular, extinction training in combination with pharmacological compounds that potentially facilitate the extinction process might become a further future alternative. On the preclinical level we have already shown that the partial N-methyl-D-aspartate (NMDA) receptor and partial agonist D-cycloserine can facilitate extinction of alcohol-seeking in rats (Vengeliene et al. 2008)—a finding that must now be translated to the human level. Another psychotherapeutic-based intervention concerns the disruption of reconsolidation processes. During reconsolidation, a retrieved memory transiently returns into a labile state and may require new protein synthesis to persist further. During this labile state, the memory is amenable to enhancement or disruption. A very recent study by Schiller et al. (2010) implies that long-lasting drug memories can possibly be updated with non-drug-related information provided during the reconsolidation window—an intervention that may also attenuate alcohol cue memories. In laboratory animals, disruption of reconsolidation can be also achieved via application of NMDA receptor antagonists (von der Goltz et al. 2009; Milton et al. 2012), and again a combination of behavioral and pharmacological treatment strategies might be the optimal approach for future studies.

We will first provide a historical overview about relapse prevention strategies. We will then review the development of disulfiram, naltrexone, and acamprosate. Today these are the drugs of choice for clinicians to treat alcohol-dependent

patients who have a strong motivation for abstinence.[1] A large phase III RCT with nalmefene, sponsored by Lundbeck, has just been completed and the preliminary results are very positive. In 2012, the approval of nalmefene is expected by the food and drug administration (FDA) and other regulatory agencies; the opioid receptor antagonist nalmefene will then be introduced to the market as the first harm reduction medication. Serotonin-reuptake inhibitors and several other classes of psychotherapeutics are not only used for the treatment of comorbidities of alcohol dependence, but also with the indirect intention of reducing drinking, craving, and relapse. These "indirect" pharmacological treatment options will not be reviewed here. Instead, we will give an overview of newly developed preclinical compounds (e.g., neramexane) and describe how they can best be translated into the clinical situation.

2 Brief Historical Overview of Relapse Prevention Strategies

Here we will concentrate on historical developments in Europe and America, although prevention strategies have been developed in China and other countries centuries ago. In a recent book entitled "Drugs for Relapse Prevention of Alcoholism" (Spanagel and Mann 2005), Griffith Edwards from London provided a comprehensive overview of the history of the prevention of relapse that is briefly recapitulated here. In the sixteenth century, punishment was the approach to diminish alcohol consumption. For example, during the reign of François I in France, an edict in the year 1536 stipulated:

> Anybody who appeared in public in the state of intoxication should on the first occasion be imprisoned on bread and water. On the second occasion chastised with birch and whip, and on the third occasion publically flogged. Should further relapses occur the delinquent was to have an ear cut off and suffer banishment.

Although the concept of punishment might have caused fear in sporadic populations of individuals experiencing drunkenness, it became outdated in the eighteenth century when a first epidemic of gin consumption permeated England. There were no regulations on gin distillation and it could be sold tax-free throughout the country. In the working class in London and other large English cities, extreme Gin inebriation provoked moral outrage and became a major societal health problem. The government was forced to react on these societal excesses and in 1750 *The Sale of Spirits Act* was enacted to reduce gin

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[1] In a few countries (e.g., Italy), γ-Hydroxybutyric acid (GHB) is a treatment option officially approved by the respective regulatory agencies and a large European trial has been initiated to further test the effectiveness of this drug. However, it should be emphasized that GBH is a street drug categorized as illegal in many countries. Clearly, GBH bears a strong potential to be abused and even in the context of being used as a substitution drug may produce multiple side effects, including the impairment of the immunological status of an abuser (Pichini et al. 2010).

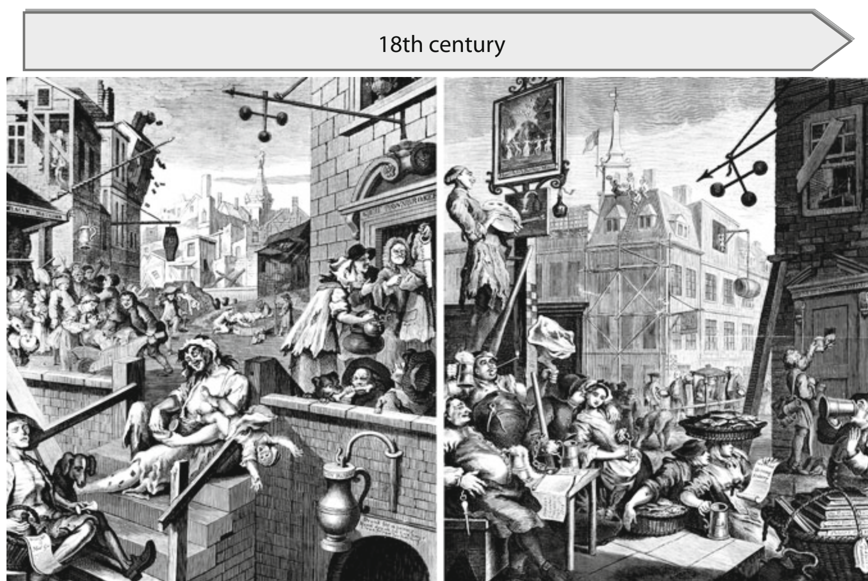

Fig. 1 William Hogarth's prints from 1751 from gin lane to beer street (The British Museum, London, UK)

consumption by raising taxes and prohibiting gin distillers from selling to unlicensed merchants. At the same time, the English painter William Hogarth (1697–1764) pictured the unpleasant consequences of alcoholism on "gin lane" and proposed that replacing gin with beer (Beer street; Fig. 1) would reduce the harm of alcohol drinking and in an imaginative ideal would even result in a society full of harmony.

Today "beer street" gets celebrated each year at the Oktoberfest in Munich, and though one might have the impression of a societal harmonically event, within the blink of an eye comes the realization of the harmful effects of excessive alcohol consumption, such as aggressive behavior, sexual disinhibition, and the devastating effects the day after. Nevertheless, William Hogarth's idea to shift from gin to beer drinking was probably the first public effort of an artist to interfere with societal issues and his prints were published in support of the Gin Act. What is really fascinating regarding the proposed shift from gin lane to beer street was the suggestion of a replacement therapy even at that early time. Today most researchers, clinicians, and also politicians are not tolerant of the idea that alcohol could be replaced by another psychoactive compound (e.g., GHB), despite the fact that this is the most successful treatment option in opiate addiction (Dole and Nyswander 1967; for a recent Cochrane Database Systematic Review, see Mattick et al. 2009). Later in this chapter we will return to the introduction of neramexane —a NMDA receptor channel blocker—as a replacement therapy for alcohol-dependent patients.

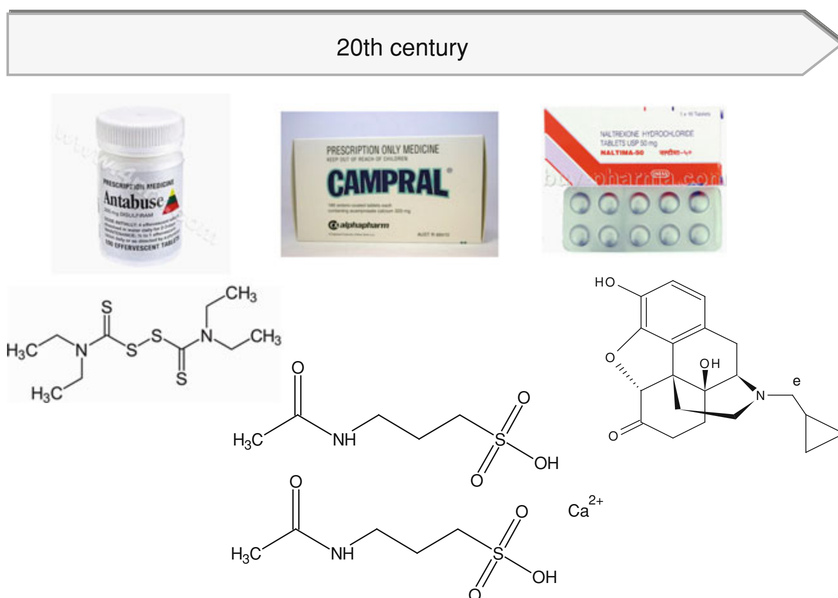

Fig. 2 The twentieth century has yielded three medications for relapse prevention in detoxified alcohol-dependent patients. Disulfiram has been introduced under the trade name Antabuse®, acamprosate as Campral®, and naltrexone as Revia® to the market

In the nineteenth century, alcoholism was more and more seen from a medical and psychological perspective. The concept emerged that excessive drinking is a learned habit, an idea that was particularly well-developed by Scottish physician Thomas Trotter (1760–1832) in his famous “Essay on Drunkenness.” This book was among the first attempts to characterize excessive drinking as a disease or medical condition, concluding that drunkenness is a disease that could be cured. This disease concept of alcoholism became the framework for the twentieth century, during which the pharmacological treatments for alcohol dependence emerged. The first modern pharmacological intervention strategy was, as often in pharmacology, discovered by accident in 1947 at the Royal Danish School of Pharmacy in Copenhagen. At that time Danish researchers Eric Jacobsen and Jens Hald were studying compounds for possible use in treating parasitic stomach infections. In a self-experiment—a kind of heroic behavior that has unfortunately disappeared from the landscape of modern work attitudes of chemists and pharmacists—they administered to themselves a small dose of one of their compounds to check for possible side effects. The next day they became very ill after having a drink. Because each of them experienced the same symptoms at the same time, they assumed that the drug and alcohol interaction was responsible for their illness. Antabuse® (disulfiram) (Fig. 2) was born and further human experiments quickly led to the conclusion that Antabuse is a drug that sensitizes the organism to ethanol, a finding published in Lancet one year after the initial observation was

made (Hald and Jacobsen 1948). These first clinical observations instigated several clinical trials in different countries and an expert panel, on request by the Canadian Medical Association delivered in the early 1950s the conclusive statement that Antabuse would prove valuable as an adjunct in the treatment of alcoholic patients but should be used only on carefully selected patients, with a full realization of its potential danger. In the last 60 years, thousands of patients have been treated with Antabuse, and the determination is that this medication reveals a mixed outcome pattern—some evidence that drinking frequency is reduced but minimal evidence to support improved continuous abstinence rates (Garbutt et al. 1999). Nevertheless, as initially suggested, in carefully selected patients with a continuous clinical monitoring, Antabuse has its value.

What does disulfiram do to the organism? Under physiological metabolic conditions, ethanol is broken down in the liver by the enzyme alcohol dehydrogenase to acetaldehyde, which is then converted by the enzyme acetaldehyde dehydrogenase to harmless acetic acid. Disulfiram interrupts this reaction at the intermediate stage by inhibiting the enzyme acetaldehyde dehydrogenase. After alcohol intake under the influence of disulfiram, the concentration of acetaldehyde in the blood may be 5–10 times higher than that found during metabolism of the same amount of alcohol alone. As acetaldehyde is one of the major causes of the symptoms of a "hangover," this produces an immediate and severe negative reaction to alcohol intake, including flushing of the skin, accelerated heart rate, shortness of breath, nausea, vomiting, throbbing headache, visual disturbance, mental confusion, etc. It was found later that disulfiram also blocks dopamine-ß-hydroxylase (Goldstein et al. 1964)—an enzyme that leads to the conversion of dopamine into noradrenaline. Disulfiram-induced inhibition of dopamine-ß-hydroxylase thus leads to an accumulation of dopamine and concurrently to a reduction of noradrenaline in peripheral and central tissues. This bi-directional action of disulfiram on monamines may provide a new rationale for the treatment of cocaine addiction (Sofuoglu and Sewell 2009).

3 Current State of Pharmacological Relapse Prevention

What is the current state in terms of pharmacological relapse prevention? Beside Antabuse®, naltrexone—and a once-monthly extended release injectable naltrexone formulation marketed under the trade name Vivitrol® (Gastfriend 2011)—and acamprosate have been approved by most regulatory bodies and are available in most countries across the globe (Fig. 2).

Cochrane Reviews, which provide the highest standard in evidence-based health care, recently published two systematic reviews in which Rösner et al. (2010a, b) summarized all studies on naltrexone and acamprosate, and concluded that naltrexone significantly reduces the risk of habit drinking with a relative risk (RR) of 0.83, whereas acamprosate significantly reduces the risk of any drinking with a RR of 0.86. A relative risk of 1 means that there is no difference between

placebo and treatment, whereas a RR < 1 means that relapse occurs less frequently in the treatment group. The effectiveness of both compounds is comparable.

Nalmefene may soon become an interesting treatment alternative. In mid-2011, a large phase III trial was concluded. In previous smaller clinical trials conducted by Barbara Mason and colleagues, nalmefene was shown to be effective in preventing relapse to heavy drinking as compared to placebo (Mason et al. 1994, 1999). Subsequently the so-called "targeted approach" was developed; i.e., subjects were instructed to take nalmefene when they believed that drinking was about to happen. In a first Finish RCT in more than 400 patients, it was shown that this targeted approach was safe and effective in reducing heavy drinking (Karhuvaara et al. 2007). A still unpublished large phase III study has also used the targeted approach with excellent effectiveness (see trial watch: Nat Rev Drug Discov 2011, 10:566, and the study leader Karl Mann, personal communication). Hence, nalmefene might be the first "pill on demand" for treating relapse behavior.

Nalmefene is a μ-opioid receptor antagonist but also exhibits in vivo antagonistic properties at the κ-opioid receptor (KOR) (Fig. 3). Researchers at the Scripps Institute in La Jolla compared the effects of nalmefene and naltrexone in alcohol-dependent rats and demonstrated that nalmefene was significantly more effective in suppressing alcohol intake than naltrexone (Walker and Koob 2008). It was suggested that this additional effect arises from a blockade of KORs, and indeed nor-BNI, a selective antagonist at this specific opioid receptor, also suppressed alcohol intake in dependent animals (Walker et al. 2011). Why would a blockade of KOR be beneficial in reducing heavy drinking? The underlying concept was developed in the laboratory of Albert Herz at the Max-Planck-Institute of Neuropharmacology in Martinsried, Germany. There it was discovered that KOR agonists produce place aversion in rats (Mucha and Herz 1985) and induce a strong dysphoric response in human volunteers (Pfeiffer et al. 1986). In view of the euphorigenic properties of μ-opioid receptor agonists, these results suggest the existence of opposing opioid systems affecting motivational, emotional, and perceptual experiences. Subsequently, it was shown on the neurochemical level that mesolimbic dopamine neurons are modulated by opposing endogenous opioid systems, whereby the dynorphin/KOR system reduces basal dopamine levels in the nucleus accumbens (Spanagel et al. 1992). These results imply that a reduced basal dopamine level in the reinforcement system is the neurochemical substrate of aversive and dysphoric behavior. Interestingly, chronic alcohol intake leads to an up-regulation of the dynorphin/KOR system in the brain of alcohol-dependent rats and humans (Shippenberg et al. 2007; D'Addario et al. 2011; Bazov et al. 2012), and prodynorphin and KOR knockouts show reduced alcohol consumption (Kovacs et al. 2005; Blednov et al. 2006; but see also Femenía and Manzanares 2012). Further evidence that an altered dynorphin/KOR system contributes to alcohol dependence comes from genetic association studies. An association of several single nucleotide polymorphisms (SNPs) in the prodynorphin and KOR gene, respectively, and alcohol dependence has been repeatedly shown (Xuei et al. 2006; Williams et al. 2007; Flory et al. 2011), and there is

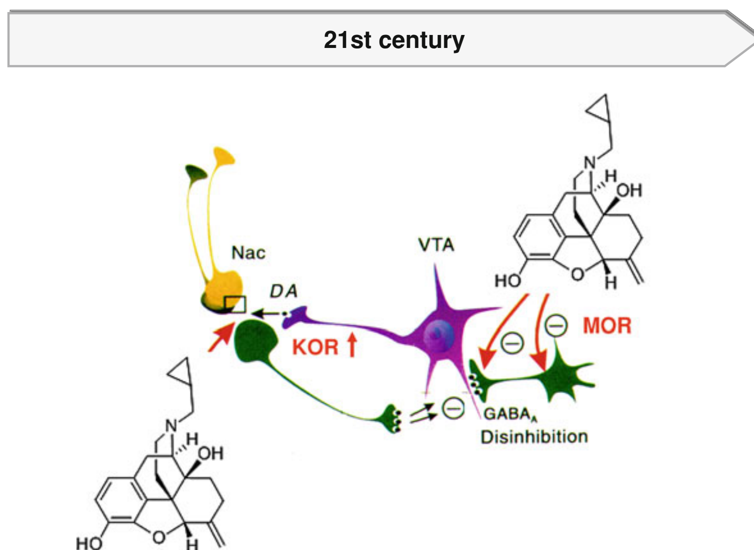

Fig. 3 Nalmefene is an opioid antagonist with a chemical structure similar to naltrexone. However, in addition to its antgonistic profile on the μ-opioid receptor (MOR) it also blocks KOR in vivo. Mesolimbic dopamine neurons are modulated by opposing endogenous opioid systems, whereby the accumbal dynorphin/KOR system reduces basal dopamine levels in this brain site. In alcohol-dependent subjects the accumbal dynorphin/KOR is upregulated and as a result a hypo-dopaminergic state ensues (Diana 2011). Nalmefene may normalize this hypo-dopaminergic state and thereby reduce alcohol craving

elevated methylation of prodynorphin CpG-SNPs associated with alcohol dependence (Taqi et al. 2011). We conclude that in the alcohol-dependent brain there is an up-regulation of the dynorphin/KOR system, and therefore a blockade of KOR may suppress the negative drive and compulsive alcohol use (for review of this concept see Wee and Koob 2010). However, the translation into the human condition has been hampered so far by the fact that there are only a few selective KOR antagonists available, all of which possess extremely long-lasting activity that limits their clinical application (Spanagel et al. 1994; for review see Peng and Neumeyer 2007). Therefore, nalmefene provides for the first time a treatment option for targeting the KOR in the human brain as it exhibits in vivo antagonistic properties at this receptor, and this effect might be the reason why nalmefene is superior in the treatment of heavy drinking in comparison to naltrexone.

4 Identification of New Drug Targets

Multiple neurochemical pathways have been identified as being involved in mediating, craving, and relapse to alcohol (Vengeliene et al. 2008, 2009; Spanagel 2009), and such knowledge provides the basis for the classical hypothesis-driven

drug target definition and subsequent compound development. Using this approach, many new targets have been identified and several new compounds are currently undergoing clinical testing (for an extensive overview, see *Drugs for Relapse Prevention of Alcoholism*, edited by Spanagel and Mann 2005; for more recent reviews, see Spanagel and Kiefer 2008; Heilig et al. 2010a, b; Edwards et al. 2011; and see a recent special issue on *Pharmacotherapy of Alcoholism* in Current Pharmaceutical Design, edited by Leggio and Addolorato 2010). Examples of this hypothesis-driven approach are described below.

Markus Heilig and George Koob postulated that corticotropin-releasing hormone (CRH) signaling via its CRH1 receptor is a key element of the neuroadaptive changes driving alcoholism and is therefore a major target for the treatment of relapse behavior, especially under stress-related conditions (Hansson et al. 2006; Heilig and Koob 2007). The role of the CRH system is further supported by a series of human genetic studies showing that specific variants of the CRHR1 gene interact with exposure to stressful life events to predict the onset of alcoholism (Treutlein et al. 2006; Blomeyer et al. 2008; Barr 2010; Nelson et al. 2010; Schmid et al. 2010). In fact, this CRHR1 gene × stress × alcohol effect seems to be the most consistent finding in the field of psychiatric genetics (with respect to gene × environment interactions). However, the application of specific CRHR1 antagonists in the translatable human situation may face two limitations. First, only a relapse risk that is driven by a high stress load may be efficiently attenuated, and second, actions of CRHR1 antagonists on hypothalamic–pituitary–adrenal (HPA) axis activity might counteract their desired therapeutic effects in alcohol-dependent patients (Sillaber et al. 2002; Molander et al. 2012). Currently a novel CRHR1 antagonist is being tested in a translational study at the (NIAAA) in Bethesda (Heilig 2011).

Another example is the dopamine D3 receptor (D3R). D3Rs exhibit the highest density in the nucleus accumbens and amygdala—brain areas that are thought to be crucial for the integration and response to the presentation of alcohol-associated cues. Furthermore, the number of these receptors is increased in addicted patients and alcohol-dependent animals. And finally, selective DA D3 antagonists show a very promising preclinical profile with no orlittle side effects (Heidbreder et al. 2005; Vengeliene et al. 2006). Moreover, these compounds have also been tested in a variety of animal models related to nicotine, cocaine, and morphine addiction with very consistent and reliable results (Heidbreder et al. 2005). In light of these findings, clinical trials have now been initiated to test the clinical significance of D3 antagonists.

From our perspective, one of the most promising candidate targets derives from the hypothesis that manipulation of the extracellular glycine pool can possibly affect relapse behavior. The glycine system has been defined as an access point for alcohol to the reinforcement system (Molander and Söderpalm 2005; Adermark et al. 2011) and thus selective glycine transporter (GlyT) blockade may affect excessive alcohol consumption via both glycine receptors and NMDARs, which also contain a glycine binding site. In fact, Org25935, a selective GlyT1 antagonist, reduced compulsive relapse-like drinking without the development of

tolerance in an animal model of alcohol addiction (Vengeliene et al. 2010; for a description of the model, see Spanagel and Hölter 1999). Importantly, these anti-relapse properties were maintained for at least 6 weeks in a treatment-free period. This persistent effect was paralleled by the reversal of altered expression levels of a set of glycinergic and glutamatergic signaling-related genes in the striatum to levels found in alcohol-naïve control rats (Vengeliene et al. 2010). In our laboratory, this is the first drug that has produced long-lasting anti-relapse effects in our animal model of alcohol addiction. Due to this finding, in combination with previous results obtained in selected high alcohol-drinking rats that demonstrated reduced alcohol intake and preference following Org25935 treatment (Molander et al. 2007), a RCT investigating the efficacy and safety of Org25935 in relapse prevention in subjects with alcohol dependence has been initiated (ClinicalTrials.gov Identifier: NCT00764660). Following the gigantic merger of Organon (who developed Org25935) with Schering–Plough in 2008, and a year later the full takeover by Merck & Co., the new enterprise implemented several strategic decisions unsupportive of CNS-related programs; consequently, the trial on Org25935 was discontinued. This is an example *par excellence* of how nonscientific decisions, based more on the shareholder value of a company than progress in medicine, are undermining the development promising medications.

Finally, many more targets have been identified and several promising candidates developed. The list includes, for example, non-peptide agonists of the nociceptin receptor as potential anti-relapse medications (Ciccocioppo et al. 2004; Kuzmin et al. 2007), neurokinin 1 receptor antagonism as a possible therapy for alcoholism (George et al. 2008), and ghrelin receptor blockade (Jerlhag et al. 2009; Landgren et al. 2011), mediated via glutamatergic control of ghrelin action at the level of the reinforcement system (Jerlhag et al. 2011). Another example is agonists at $GABA_B$ receptors, such as baclofen (Colombo et al. 2004; Addolorato et al. 2007)—a drug described by French cardiologist Olivier Ameisen in the best-selling book "The End of my Addiction" as the magic bullet. Unfortunately, only future RCT will reveal whether these and other pharmacotherapies will benefit alcohol-dependent patients. We again refer the reader to a series of excellent reviews describing these pharmacological alternatives (Spanagel and Kiefer 2008; Heilig et al. 2010a, b; Edwards et al. 2011; and see a recent special issue on *Pharmacotherapy of Alcoholism* in Current Pharmaceutical Design, edited by Leggio and Addolorato 2010) and would like to reemphasize that there is no other field in psychiatric research that has yielded as many promising approaches in target definition and drug development than that for alcohol addiction.

Alternatively, the variety of putative targets also demonstrates that alcohol affects many neurotransmitter systems (Vengeliene et al. 2008; Spanagel 2009), and it is unlikely that specifically targeting one access point of alcohol to the brain reinforcement system will benefit a large proportion of alcohol-dependent patients. Therefore, an additional benefit might arise from the combination of different compounds. In fact, two clinical studies have demonstrated that a combination of naltrexone and acamprosate may be more effective than either drug alone (Kiefer et al. 2003; Feeney et al. 2006). However, these findings were not replicated in the

COMBINE study, which included 1,383 alcohol-dependent patients treated with a combination of both compounds as well as behavioral interventions (Anton et al. 2006). The reason for this negative finding is most likely due to a recruitment bias of subjects with moderate severity and early stages of alcoholism, which defines a group of patients that is more responsive to naltrexone than acamprosate treatment (Karl Mann, personal communication). In conclusion, we suggest that a "cocktail" of different compounds will further improve the treatment situation. However, the question remains whether the pharmaceutical industry will invest the money for further cost-intensive phase II–III studies. Given their hesitant efforts in the past and their economically motivated lack of interest in testing drug cocktails, joint efforts of academia, and the pharmaceutical industry will be required in the near future.

5 Convergent Genomic Analysis for New Drug Target Definition

Another potential for drug target definition arises from convergent genomic analysis. A genetic component of vulnerability to alcohol addiction has long been established. The heritability of alcoholism lies at approximately 50–60% (Goldman et al. 2005). Although it is a complex disorder and the contribution of single genes to the clinical phenotype(s) of addictive behavior is rather small, genome wide analysis of variants contributing to increased vulnerability for alcohol addiction may yield new targets. One approach that we took—under the leadership of Gunter Schumann of the Institute of Psychiatry in London—was conducting a genome-wide association study (GWAS) on high alcohol consumption in combination with animal studies (for consilience of alcohol drinking behavior in animals and humans, see Kiefer and Spanagel 2006 and Leeman et al. 2010). Indeed, human genetic data can be further enriched by information from animal studies. A new translational approach for the integration of data sets that derive from forward genetics in animals and genetic association studies, including GWAS in humans, is referred to as convergent functional genomics. We recently obtained new targets by applying this approach (Treutlein et al. 2009; for confirmation, see Frank et al. 2012). The aim of forward genetics in animals and association studies in humans is to identify mutations (e.g., SNPs) that produce a certain phenotype; i.e., "*from phenotype to genotype*." The repertoire of forward genetics in animals includes the generation of random mutations in an organism, either by radiation, or chemical mutagens such as N-ethyl-N-nitrosourea, and then through a series of breeding of subsequent generations, isolating individuals with a phenotype relevant for addictive behavior (Pawlak et al. 2008). Most powerful, however, in terms of forward genetics, is combined quantitative trait loci analysis and differential gene expression profiling in recombinant inbred rodent lines or animals genetically selected for a specific phenotype, such as, for example, high versus low alcohol consumption (Spence et al. 2005; Ehlers et al. 2010). Baysian approaches allow combining such animal genomics data with GWAS information

from a similar addiction-relevant human phenotype, thereby enhancing the explanatory power of genetic studies. Within the context of a huge consortium, we performed such a convergent functional genomics analysis. Twelve population-based samples comprising 28,188 individuals screened for their alcohol intake per day in gram intake per kilogram bodyweight, and an almost equally large replication genotyping sample with 21,185 individuals of European ancestry, were used to identify genetic loci associated with high alcohol intake (Schumann et al. 2011). Out of approximately 2.5 million directly genotyped or imputed SNPs, we found a few variants with genome-wide significance. One hit drew our attention, an identified SNP in or near the *Ras protein-specific guanine nucleotide releasing factor 2* (*RASGRF2*) gene that may indeed be of high relevance for excessive and compulsive alcohol consumption, but only in males. The *RASGRF2* is a glutamate transmission-related gene that couples NMDA receptors to the activation of the Ras-ERK signaling cascade. The role of NMDA receptors and Ras-ERK signaling in alcohol-induced plasticity is well-established. In recent years, the role of the Ras-ERK pathway and downstream gene expression has extensively been investigated in the striatum, using both pharmacological and genetic approaches. The conclusion of these studies is that an aberrant hyperactivation of Ras-ERK appears to be a key pathogenic factor for addictive behavior (Schroeder et al. 2008; Fasano and Brambilla 2011). In order to study the functional relevance of this gene, we used *RASGRF2* knockouts and studied them in free-choice drinking conditions with increasing concentrations of ethanol. The knockouts consumed significantly less alcohol, especially at pharmacologically relevant concentrations. Furthermore, consistent with the aforementioned convergent functional genomics analysis, this phenotype effect occurred only in male mice, as female mutants drank equal amount of acohol as wild-type control animals. These findings have two important implications. First, the Ras-ERK pathway may be a new target for treating compulsive alcohol drinking, and compounds that result in a blockade of this signaling pathway already exist. Second, it is astonishing that a hypothesis-free approach yielded a hit in a gene that is of critical importance for glutamate-NMDA receptor signaling, as one of the major theories in the alcohol addiction research field predicts that blocking a potential hyper-glutamatergic state in the addicted brain may reduce alcohol craving and relapse (Tsai et al. 1995; Krystal et al. 2003; Gass and Olive 2008; Spanagel and Kiefer 2008; Spanagel et al. 2010).

6 Treating a Hyper-Glutamatergic System

In recent years, the glutamate theory of alcoholism and addictive behavior has emerged as a major theory in the addiction research field. In a seminal publication, Lovinger et al. (1989) demonstrated that NMDA receptor function was inhibited by ethanol in a concentration-dependent manner over a range of 5–50 mM, a range that produces intoxication. Further research using site-directed mutagenesis experiments identified putative binding sites for ethanol molecules at the NMDA

receptor (for review, see Spanagel 2009). Thus, the first level of interaction of alcohol with brain function concerns the NMDA receptor (among other primary targets of ethanol in the brain; for an overview, see Vengeliene et al. 2008). The NMDA receptor is a ligand-gated ion channel with a heteromeric assembly of NR1, NR2 (A–D), and NR3 subunits. The NR1 subunit is crucial for channel function, the NR2 subunits contain the glutamate binding site, and the NR3 subunits have a modulatory function on channel activity, especially under pathological conditions. Several transmembrane domains of the NR1 and NR2A subunits have putative alcohol binding sites. Beside this direct interaction with the NMDA receptor, alcohol also affects the glutamatergic system at the synaptic and cellular level, and it is further proposed that through various neuroadaptive responses that restore homeostasis, chronic alcohol consumption may lead to an enhanced activity of the glutamatergic system in alcohol-dependent individuals (Tsai and Coyle 1998). This glutamate-induced hyperexcitability within the CNS is uncovered during alcohol withdrawal. Acute alcohol withdrawal responses, which typically occur after discontinuation of prolonged and excessive alcohol ingestion, contribute to disease progression. Some of these neuroadaptations are transient, but the persistent changes remain during protracted abstinence and underlie vulnerability to relapse (Sommer et al. 2008; Heilig et al. 2010a, b). Acute withdrawal is associated with increased central glutamatergic transmission. More than 10 published reports employing brain microdialysis experiments in alcohol-dependent animals have consistently demonstrated augmented extracellular glutamate levels in various brain sites that correlate perfectly with the intensity of the withdrawal response (Rossetti and Carboni 1995; De Witte et al. 2003; Gass and Olive 2008). Furthermore, after repeated cycles of withdrawal, this hyper-glutamatergic response is progressively augmented (Dahchour and De Witte 2003; Gass and Olive 2008; Chefer et al. 2011). Augmented glutamatergic activity also occurs during conditioned withdrawal responses (Cole et al. 2000; Dahchour and De Witte 2003) and may therefore contribute to craving and relapse behavior. In a recent study by Gass et al. (2011), an increase in extracellular glutamate transmission in the nucleus accumbens was found during cue-induced alcohol-seeking behavior. In this study, rats were trained to self-administer either alcohol or food pellets. Each reinforcer was accompanied by the presentation of a light/tone stimulus. Following stabilization of responding for alcohol or food reinforcement, and subsequent extinction training, animals were implanted with glutamate oxidase-coated biosensors and underwent a cue-induced reinstatement testing period. Extracellular levels of glutamate were increased in the nucleus accumbens core during cue-induced reinstatement of alcohol-seeking behavior, an effect that did not occur during conditioned cue-induced food-seeking (Gass et al. 2011). These results indicate that increases in glutamate transmission in the nucleus accumbens core may be a neurochemical substrate of cue-induced alcohol-seeking behavior. In conclusion, these findings suggest that persistent neuroadaptations in glutamatergic functioning may play a key role in the pathophysiology of alcoholism. This provides the rationale for using anti-glutamatergic compounds such as acamprosate for relapse prevention—the suggested mode of action of acamprosate

is that it dampens a hyper-glutamatergic state through an as yet unidentified mechanism (Spanagel and Kiefer 2008).

Recently, the glutamate theory has also been tested at the human level. In human alcoholics undergoing acute withdrawal, increased glutamate levels were identified in the anterior cingulate cortex, a brain region known to be critically affected by chronic alcohol intake, using high-resolution magnetic resonance (MR) spectroscopy (Hermann et al. 2011). Furthermore, these increased glutamate levels were significantly higher in treatment-seeking alcoholic patients as compared to healthy control subjects. Importantly, the glutamate signal was also correlated with the severity of the withdrawal reaction. After two weeks of abstinence, glutamate levels returned to levels similar to those detected in controls. By means of ultra high field strength generated by a 9.4 Tesla (T) animal scanner, the authors also observed a similar time course of the glutamate signal in the rat medial prefrontal cortex, a region comparable to the anterior cingulate cortex in humans. There were striking similarities between the human and the rat brain in the baseline measurements and dynamics of the glutamatergic system during the time course of acute and protracted alcohol withdrawal, demonstrating the validity of MR spectroscopy as a translational tool (Hermann et al. 2011). In yet another MR spectroscopy study, the effects of acamprosate in detoxified alcohol-dependent patients on central glutamate levels were assessed (Umhau et al. 2010). Thirty-three patients who met the diagnostic criteria for alcohol dependence and were admitted for medically supervised withdrawal from ongoing alcohol use were included in this study. The design was a 4-week, double-blind, placebo-controlled experimental medicine study, with MR spectroscopy measurements using a 3 T scanner obtained on days 4 and 25. Fifteen patients received acamprosate and 18 received placebo. There was a highly significant suppression of central glutamate levels across time by acamprosate, demonstrating for the first time that this anti-relapse medication may dampen a hyper-glutamatergic state in the brain of alcoholics.

The mode of action of acamprosate, a reduction of a hyper-glutamatergic state, has been thoroughly demonstrated in animal models. Several mutant mouse lines have been identified that exhibit a hyper-glutamatergic state (Spanagel et al. 2005; Lee et al. 2011). One of these mouse models involves the clock gene *Period* (*Per*). Interestingly, the mouse *Per2* gene modulates, via the glutamate transporter GLAST, extracellular glutamate levels, resulting in hyperexcitability and enhanced consumption of alcohol (Spanagel et al. 2005). If acamprosate acts via a hyper-glutamatergic state to reduce excessive drinking, it can be inferred that mice lacking the *Per2* gene should be especially sensitive to acamprosate treatment. Indeed, following repeated acamprosate administration, mutant mice showed decreased alcohol consumption as well as a normalization of extracellular glutamate levels in the nucleus accumbens (Spanagel et al. 2005). In a follow-up study by Brager et al. (2011a), it was confirmed that alcohol intake and preference was much greater in *Per2* mutants than in wild-type mice. The authors of this study examined diurnal alcohol drinking activity more closely and found that the suppressive action of acamprosate on alcohol intake was due to a reduction in the

amplitude and number of daily drinking bouts and not due to changes in diurnal alcohol drinking patterns. To determine brain sites responsive to acamprosate, a brain mapping study with acamprosate microimplants was conducted (Brager et al. 2011b). Mice were given voluntary access to alcohol followed by a period of abstinence, after which the alcohol deprivation effect (ADE)[2] was measured. Four days before alcohol was reintroduced, mice received bilateral blank or acamprosate-containing microimplants releasing approximately 50 ng/day into the ventral tegmental area, nucleus accumbens, or suprachiasmatic nucleus. The hippocampus was targeted as a negative control site. Acamprosate in all areas, except the hippocampus, suppressed alcohol intake and preference during the ADE. These data demonstrate that the suppression of alcohol intake and preference by acamprosate during relapse-like drinking is mediated through actions within major reward and circadian sites (Brager et al. 2011b). Another mouse model exhibiting increased glutamate levels in the nucleus accumbens that are paralleled by increased alcohol drinking behavior involves deletion of the type 1 equilibrative nucleoside transporter (Ent1). Similar to the results in *Per2* mutant mice, acamprosate significantly reduced alcohol drinking in *Ent1* mutant mice while having no effect in wild-type littermates (Lee et al. 2011). Basal and acamprosate-treated accumbal metabolite profiles of *Ent1* mutant mice and wild-type mice were further assessed using in vivo 16.4 T MR spectroscopy. Lee et al. (2011) found enhanced basal glutamate levels in the nucleus accumbens in *Ent1* mutant mice compared to wild-type mice. They also found that acamprosate treatment significantly reduced glutamate levels in the mutants, while glutamate levels in wild-type mice remained unaltered. In summary, these mouse models (*Per2* and *Ent1*) provide a clear link between a hyper-glutamatergic state and excessive alcohol consumption. They further demonstrate that acamprosate acts only on a hyper-glutamatergic state. Future research should address whether human *Per2* gene variants may predict enhanced vulnerability to alcohol dependence and augmented central glutamate levels. If this is the case, screening of human *Per2* gene variants could be used to enhance the number of acamprosate responders. In fact, an association study has identified a specific genetic variation of the human *Per2* gene that is associated with high alcohol consumption (Spanagel et al. 2005).

Within the framework of the glutamate theory of alcoholism, it is proposed that NMDA receptors, neuronal glutamate release properties, and other components of the glutamate system are involved in the etiology of alcohol addiction. Given that glutamatergic components are crucial in disease progression, we recently performed a hypothesis-driven gene expression profiling in alcohol drinking rats (Vengeliene et al. 2010) wherein we designed a custom-made microarray containing glutamate transmission-related genes. With this chip we were able to

[2] Relapse-like behavior in animals is characterized by the ADE. Following a period of alcohol abstinence, animals considerably but temporally increase voluntary alcohol intake as compared to basal consumption levels. Following repeated deprivation phases, the ADE is characterized by an increased and compulsive demand for alcohol that clearly dissociates from normal drinking behavior (Spanagel and Hölter 1999; Vengeliene et al. 2009).

screen for approximately 200 selected genes, including sets of presynaptic genes (vesicles, docking, and exocytosis-associated genes), postsynaptic genes (receptors, anchoring, signal transduction, and transcription associated genes), and perisynaptic genes (glial transporters and other associated genes). This approach of hypothesis-driven gene expression profiling greatly reduces the number of multiple comparisons relative to a whole transcriptome analysis and thereby provides increased statistical power (Gebicke-Haerter 2005). The targeted gene expression profiling was conducted in brain tissue derived from the striatum of an alcohol-naïve group versus that of a group with excessive alcohol consumption for more than 1 year. Interestingly, of 202 glutamate-transmission-related genes in the striatum, 168 genes showed significant alteration following long-term excessive alcohol consumption. Extensive qRT-PCR analysis validated these findings (Vengeliene et al. 2010). In humans, post-mortem striatal brain tissue from diseased alcoholics demonstrates that various glutamatergic markers are similarly altered, and a systematic analysis of glutamate transmission-related genes in alcohol-dependent patients revealed that NR2A and metabotropic glutamate receptor 5 (mGluR5) have the highest relevance for human alcohol dependence among the genes selected, with odds ratios of 2.35 and 1.69, respectively. In particular, a NR2A variant was associated with positive family history, early onset of alcoholism, and maximum number of drinks in adults as well as harmful drinking patterns in adolescents (Schumann et al. 2008; for replication study, see Domart et al. 2011). To further investigate the association of variation in a set of genes from the NMDA receptor complex and signaling with alcohol dependence, a gene-set analysis was recently conducted (Karpyak et al. 2011). Rather than testing for association with each SNP individually, which typically results in power too low to detect small effects of multiple SNPs, gene-set analysis applies a single statistical test to evaluate whether variation in a set of genes is associated with the phenotype of interest. Almost 1,000 SNPs from 13 genes were examined, and demonstrated a significant association with alcohol dependence for the global effect of variation in the NMDA receptor complex and signaling (Karpyak et al. 2011).

In summary, these findings provide the rationale for using NMDA receptor blockers or other anti-glutamatergic compounds for relapse prevention. A variety of modulators of NMDA receptor activity have recently been considered in the search for pharmacotherapeutic agents that may be useful in the treatment of alcoholism. In particular, neramexane—a noncompetitive NMDA receptor channel blocker—has been proposed as a promising drug for relapse prevention, with many preclinical findings consistent with this proposal. For example, like memantine, neramexane dose-dependently substitutes for the ethanol cue in a discrimination task (Hundt et al. 1998; Hölter et al. 2000), suppresses ethanol withdrawal seizures (Bienkowski et al. 2001; Kotlinska et al. 2004), and reduces responding for ethanol under operant conditions (Bienkowski et al. 1999). Furthermore, neramexane prevents the acquisition and expression of ethanol-induced conditioned place preference (Kotlinska et al. 2004), inhibits the expression of ethanol-induced sensitization (Kotlinska et al. 2006), and most

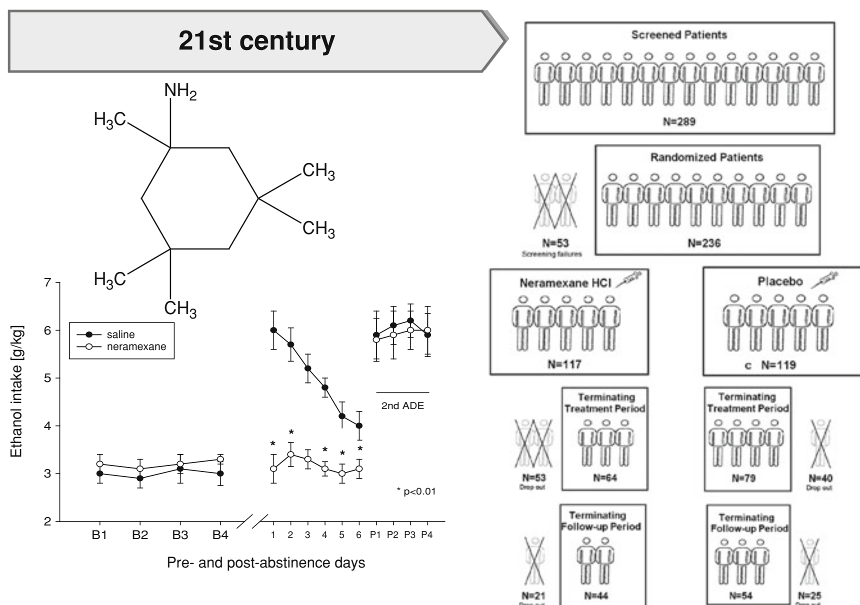

Fig. 4 Neramexane has a memantine-like structure and completely suppresses the ADE in long-term alcohol drinking rats. However, the drug has to be "on board" to exhibit an anti-relapse effect. Thus, if previously neramexane-treated rats undergo a second ADE without further having ongoing treatment they will relapse to the same extend as control rats treated with vehicle (*lower left panel*). The *right panel* shows the design of a recently completed RCT with neramexane. Participants were 236 men who had been diagnosed with alcohol dependence according to the International Classification of Diseases (ICD-10). They were included after inpatient detoxification. After eligibility had been established according to inclusion and exclusion criteria, patients were randomly assigned to either neramexane (2 × 20 mg per day) or placebo both given orally in identical size and color. Twelve weeks of double-blind outpatient treatment with weekly visits up were employed. This treatment phase was followed by a medication-free 12-weeks follow-up period with visits every second week. Drinking behavior of patients was classified at each visit as abstinent, relapsed, or nonattendant. In total, 98 patients terminated this follow-up period

importantly suppresses the ADE when administered chronically either via either osmotic minipumps or repeated injections (Hölter et al. 2000; Vengeliene et al. 2005), consistent with the effects of memantine (Hölter et al. 1996). In concert with its neuroprotective potential on alcohol-induced brain damage, neramexane has a promising profile for the prevention of several consequences of alcohol abuse (Bachteler and Spanagel 2005; Rammes and Schierloch 2006).

Neramexane was tested against placebo in detoxified alcohol-dependent subjects in a multicentre RCT. The study, led by Gerhard Wiesbeck and including 19 centers specialized for the treatment of alcoholism in Austria and Germany, screened 289 patients. Of this pool, 236 were randomized to either the neramexane

group ($n = 117$; 2×20 mg per day) or the placebo group ($n = 119$). After 12 weeks of double-blind treatment, patients treated with neramexane had no benefit in terms of continuous abstinence when compared to the patients treated with placebo (Fig. 4).

A reason for this lack of effect may have been the low doses administered, as relatively high doses of the drug should be administered in the context of its use as a substitution therapy. Alterations in NMDA receptor subunit composition in alcohol-dependent subjects may have also contributed to a lack of effect. NMDA receptors composed of NR1/NR3A subunits exhibit a reduced sensitivity to channel blockers compared with NR1/NR2A receptors (Chatterton et al. 2002). In line with this conclusion, post hoc analysis on the relationship between neramexane plasma levels and the primary parameter of efficacy revealed that patients with high neramexane plasma levels had a higher rate of continuous abstinence after 12 weeks of treatment than matched placebo patients.

We conclude that high-dose treatment with neramexane results in an effective anti-relapse treatment. Two hundred and fifty years ago, William Hogarth suggested the idea of replacement therapy for gin drinkers via the use of "the less harmful beer consumption." We now have for the first time the availability of a drug that may be successful as a replacement therapy. However, neramexane may only act as a substitution therapy in alcohol-dependent patients when sufficient doses of the drug are administered.

7 Concluding Remarks and Future Perspective

Major conclusions and some future perspectives of this review are:

(i) Learning from other addictive substances is very helpful—e.g., substitution therapies as applied to opiate addiction for decades could also be translated to alcoholics. In this respect, the merger of the NIAAA and the NIDA (Kaiser 2010) will produce synergistic knowledge.

(ii) The glutamate theory of alcohol addiction provides a convincing framework for the use of NMDA receptor antagonists as substitution drugs for alcohol-dependent patients, especially the channel blocker neramexane or memantine. Both compounds have a fast on/off kinetic at the NMDA receptor channel and thereby produce less side effects than other compounds, and are promising substitution drugs provided very high doses are applied.

(iii) A combination of behavioral and pharmacological therapies might be the optimal approach for future treatment strategies—one promising example is pharmacological disruption of reconsolidation processes of alcohol cue memories. In the process of reconsolidation, a retrieved memory transiently returns into a labile state and requires new protein synthesis to persist further. During this labile state, the memory is amenable to enhancement or disruption. It has been shown that pharmacological disruption of the reconsolidation of alcohol-associated memories can be achieved by use of protein synthesis

inhibitors and NMDA receptor antagonists, and thus may provide a potential new therapeutic strategy for the prevention of relapse in alcohol addiction. Two NMDA receptor antagonists are currently being studied to this effect: memantine and the noble gas xenon. Both substances are already approved in several countries for different indications with a limited side effect profile.

(iv) Given that many neurotransmitter systems are affected by chronic alcohol consumption, numerous druggable targets have been identified; consequently, a "cocktail" of different compounds will likely further improve the treatment situation. For example, combining naltrexone and acamprosate produces a better outcome in relapse prevention than either drug on its own (Kiefer et al. 2003).

(v) In silico psychopharmacology, such as drug repurposing (Andronis et al. 2011), will yield new medications.

(vi) One size does not fit all. We have not discussed new pharmacogenetic findings that will eventually assist in individualized pharmacotherapy. Pharmacological treatment response is indeed influenced by genetic polymorphisms in drug target genes. For example, it has recently been demonstrated that a functional SNP in the μ-opioid receptor gene predicts naltrexone efficacy as measured by relapse behavior (Oslin et al. 2003; for a first meta-analysis, see Chamorro et al. 2011). Preliminary results indicate that genetic variations in GATA4 might influence relapse and treatment response to acamprosate in alcohol-dependent patients via modulation of atrial natriuretic peptide plasma levels (Kiefer et al. 2011). These results may help identify alcohol-dependent patients with an increased risk of relapse and who may better respond to acamprosate treatment. For those who are interested on the topic of pharmacogenetic approaches to the treatment of alcohol addiction, we refer the reader to an excellent recent review provided by Heilig et al. (2011; see also Sturgess et al. 2011).

(vii) Finally, the whole organism has to be taken into consideration to provide the best therapy for our patients.

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The Challenge of Studying Parallel Behaviors in Humans and Animal Models

David N. Stephens, Hans S. Crombag and Theodora Duka

Abstract The use of animal models is essential in carrying out research into clinical phenomena such as addiction. However, the complexity of the clinical condition inevitably means that even the best animal models are inadequate representations of the condition they seek to mimic. Such mismatches may account for apparent inconsistencies between discoveries in animal models, including the identification of potential novel therapies, and the translation of such discoveries to the clinic. We argue that it is overambitious to attempt to model human disorders such as addiction in animals, and especially in rodents, where "validity" of such models is often limited to superficial similarities, referred to as "face validity" that reflect quite different underlying phenomena and biological processes from the clinical situation. Instead, we suggest a more profitable approach may be to identify (a) well-defined intermediate human behavioral phenotypes that reflect defined, limited aspects of the human clinical disorder, and (b) to develop animal models that are homologous with those discrete human behavioral phenotypes in terms of psychological processes, and underlying neurobiological mechanisms. Examples of current weaknesses and suggestions for more limited approaches that may allow better homology between the test animal and human condition are made.

Keywords Intermediate behavioral phenotypes · Face validity · Alcohol addiction · Reward · Self-administration · Subjective reports · Conditioning

D. N. Stephens (✉) · H. S. Crombag · T. Duka
School of Psychology, University of Sussex, Falmer,
Brighton, BN1 9QG, UK
e-mail: d.stephens@sussex.ac.uk

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Contents

1 Introduction 612
2 Theoretical Context 614
3 Rewarding Effects of Alcohol 614
  3.1 Self-Administration 616
  3.2 Conditioned Place Preference 617
4 Models of Conditioning in Addiction 620
5 Studying Drug-Related Conditioning in Animals 622
  5.1 Conditioned Reinforcement 622
  5.2 Pavlovian Approach 623
  5.3 Pavlovian-Instrumental Transfer 624
  5.4 Potential of Conditioning Measures as Biomarkers 625
6 Subjective Experience 625
7 Alcohol and Impulsivity 626
8 Withdrawal from Alcohol 629
9 Binge Drinking and Withdrawal 630
10 Adolescent Drinking 634
11 Conclusion 635
References 636

1 Introduction

A challenge for biomedical researchers is to model human conditions in animals, so that questions that, for ethical or technical reasons, are not readily approached in humans can be addressed experimentally. Within the field of addiction, and in the context of the present article on alcohol abuse, there is a continuing need to understand the causes of addiction, the consequences of alcohol abuse, and, not least, how pharmacological and behavioral manipulations may be used to prevent or treat addictions or at least to diminish the negative consequences of long-term alcohol abuse. Yet, despite decades of research and the investment of considerable sums of money, we have only a glimmer of hope for such a rational, empirical and theory-based treatment of addiction. The aim of this article is to ask why, given the wealth and ingenuity of research, is this case? And what can we do differently to ensure future success?

Addiction science has made much headway over the past decades. In contrast to 25 years ago, we now know the principle targets for most drugs of abuse (though alcohol remains a problem child), we have a panoply of theoretical approaches to account for different aspects of addiction, and perhaps different kinds of addiction, and we have implicated a number of brain areas in the psychological and biological functions required by these theories. Although we have only a partial knowledge of workings within and between those brain areas, and how their function changes during the addiction process, important progress is being made at the systems, cellular and genomic levels. And yet we appear to be no closer today

to effective treatments for addictions than we were twenty years ago. For example, our ever-more sophisticated knowledge of the role of dopamine and its various receptors in motivational processing has not led to the development of useful therapies based on the actions of this, or closely related neurotransmitter systems. The realization that glutamatergic processes play a critical and complex role in the neuroplasticity underlying the development of addictions has not been followed by therapies targeting these processes. Rather, the currently most useful pharmacological agents are aimed, not at modifying the neurobiological and/or psychological processes underlying addiction, but simply provide substitutes, albeit with important social and medical advantages, for the drugs whose addiction they are used to treat. Methadone has been used in this way for many years, not to treat heroin addiction, but to divert it. In the UK there are the beginnings of a debate about developing substitutes for alcohol that do not induce liver damage, reflecting, in part, a sense of hopelessness about the possibility of actually treating alcoholism. And neurobiology and pharmacology are not the only failures in this scenario. Despite the wealth of evidence and theoretical understanding of the importance of drug-associated cues in initiating drug seeking and craving, therapeutic behavioral strategies aimed at weakening their emotional and motivating impact have proven largely ineffective in treating addiction and relapse vulnerability.

These frustrating facts indicate a basic weakness in our current understanding of the psychological and neurobiological bases of addiction, and raise fundamental questions regarding our approaches. Many (but certainly not all) of the ideas on which our therapeutic proposals have been based are derived from animal models, and an easy conclusion for the lack of success in developing treatments might be that such models are not only inadequate (no model can be a complete replica of the state it attempts to mimic), but are even misleading. A critic may opine that preclinical scientists in the addiction area have promised much (especially in the Introductory sections of our grant applications), but delivered little of practical value. Why should that be?

Perhaps a major reason is that animal researchers are simply too ambitious (and/or even simplistic) in what they are trying to achieve. Animals do not develop alcoholism; neither do they abuse alcohol. Thus, an approach that attempts to model either of these human disorders in animals is inevitably doomed. For that reason, a more sensible, though less ambitious approach may be to identify aspects of behavior that are fundamental to the addiction process (biomarkers, or intermediate behavioral phenotypes; Duka et al. 2010), to establish human experimental laboratory models and procedures that probe those behaviors that map well to select aspects of addiction, and then to establish animal models that are homologous with (not simply analogous to) the human models.

Of course, the relationship between these steps is a logical, not a chronological one, and it may well be that the discovery of an aberrant behavior in the laboratory animal precedes the establishment of a human laboratory procedure modeling a specific aspect thought critical in the addiction process. Whatever the sequence of events, there are two logical steps in the process; first, the behavior under study

must reflect a functional aspect of the addiction process that is recognized (empirically and/or theoretically established) as contributing to addictive behavior, and second, the animal model needs to be homologous with the human laboratory model. A corollary of the approach is that it requires the animal researcher to eschew the approach that identifies only superficial similarities (analogies) between the behavior in the lab and the clinical reality (face validity), in favor of studying behaviors that are theoretically robustly related to the addictive process. And there lies the rub; we do not yet have a universally accepted theory, or even broad conceptual framework, of addiction and it may well be that no single theory can be adequate to account for such a complex and multifactorial phenomenon. Nevertheless, it behoves the researcher to develop and place their own behavioral model within a robust theoretical framework, something that preclinical researchers in the addiction field, as well as other mental health areas, do seldom attempt.

This issue is receiving increasing attention, to the extent that an entire recent issue of the journal *Addiction Biology* addresses the problem of reconciling human and animal alcohol-related phenotypes. The reader is referred to the several excellent articles therein (Crabbe 2010; Dick et al. 2010; Ehlers et al. 2010; Heilig et al. 2010; Leeman et al. 2010; Sher et al. 2010; Stephens et al. 2010) for an in-depth analysis of some of the topics that can only be dealt with superficially in the present article. However, for the most part, these articles are designed to address the second requirement, the issue of homology between human and animal models. The issue of whether the behavior under study reflects a functional aspect of the addiction process that is recognized (empirically and/or theoretically established) as contributing to addictive behavior, is a deeper one, for which there is no complete answer at present.

2 Theoretical Context

From the aforementioned, it should be clear that the interpretation of animal experiments, or for that matter, any experiment, is theory dependent. As noted by the sociologist Homans 1950, “there is nothing more lost than a loose fact” and we would extend this thought to “there is nothing more lost than a loose model”. Models and methods that are held to be theory-free are unlikely to provide a close analogy, let alone homology, between the clinical reality and the human experimental or animal laboratory. And while such analogous models may have the appearance of completeness and relevance to the outside world (and often to the more high-profile and broadly read scientific journals), it is our strong view that if they are to serve as useful models to explore the fundamental underlying substrates and mechanisms, animal and human experimental tests need to be based on theoretically sound principles that establish homology between the processes and mechanisms (psychological or neurobiological) probed by our laboratory tests and those underlying the clinical condition. Moreover, it is worth noting that certain

tests and procedures in the laboratory that seek to probe such homologous processes, may nevertheless look quite dissimilar on the surface. However, the mere presence or absence of “face value” is neither sufficient nor necessary to justify inclusion or exclusion of the animal or human experimental model in our empirical analyses of drug and alcohol-related behaviors. It is against this conceptual background that we discuss and evaluate the empirical study of alcohol abuse -and addiction in the animal and human experimental laboratory.

3 Rewarding Effects of Alcohol

A classical view of alcohol abuse is that alcohol is taken because it is in some way rewarding. However, the nature of the rewarding effect is far from clear and may differ across individuals. In practice, reward value probably represents an aggregate measure resulting from the experience of “euphoria” and “feelings of high”, as well as those more related to relaxation, satisfaction and fulfilment, relief from tension and craving, etc. Early reports from studies with alcohol and other abused drugs indicated that the drug reward-value assessments can differ substantially among individual subjects (Schuckit 1984,1994). The extent, then, to which ethanol “reward” represents a useful concept for assessing alcohol’s effects and/or the success of potential treatments of alcohol abuse, must remain questionable.

A recent study proposed a 3-factor model, capturing the dimensions (1) stimulation and other pleasant effects, (2) sedative and unpleasant effects and (3) alleviation of tension and negative mood (Ray et al. 2009). The subjective evaluation may be characteristic for the individual, and may represent a heritable trait (Viken et al. 2003), but it is also clear that the effect experienced depends upon dose and pharmacokinetic time course. Thus, the euphorigenic effects of alcohol are often associated with rising blood alcohol levels (e.g. Erblich et al. 2003; Martin et al. 1993) while declining levels are more likely accompanied by sedation (Earleywine 1994a, b; Erblich et al. 2003). Within the clinic, it is accepted that certain individuals drink for the euphoric effects of alcohol, while others drink to alleviate anxious moods (Booth and Hasking 2009; Goldsmith et al. 2009). This variability in response, both between and within individuals, poses a problem for scientists seeking to model the rewarding effects of alcohol in animals in terms of which strain to use, and whether to model the effects of the rising or declining phases of blood alcohol levels.

Worse, measures of alcohol reward in humans typically depend upon subjective self-assessment (self-report) of mood states, something that has no translational equivalent, and thus no meaning, in the animal laboratory. Within the human laboratory, several questionnaire-type tools are available, of which the best known is the profile of mood states (POMS; (McNair et al. 1971)). However, even within the human literature, the results obtained from application of different rating scales are not entirely consistent (Ray et al. 2010), and there is long-standing evidence to indicate that human subjects have poor conscious access to, and/or cannot reliably

report to us about their affective states (Nisbet and Wilson 1977). This fundamental problem is even more evident when researchers have to rely on retrospective reports as even brief delays between the actual experience and reporting produce pronounced biases (Schwarz 2007). Where then does the animal researcher start to model human assessments of reward?

3.1 Self-Administration

Partly for the foregoing reasons, human instrumental self-administration procedures have been used extensively as a proxy-measure of the rewarding qualities of addictive drug and these procedures have been used with different drugs of abuse including morphine and cocaine, though we are aware of only a limited literature on human self-administration studies with ethanol (Bigelow et al. 1975; Griffiths et al. 1975, 1976). However, for those drugs for which such studies are available, it is important to note that self-administration measures of reward value do not always correlate with subjective reports of liking, indicating that these measures do not reflect a single (or perhaps even a related) phenomenon (Comer et al. 2008). Furthermore, these types of findings seem to reinforce earlier work reporting that people will self-administer doses of morphine or cocaine that produce no reported subjective effects of any kind (Fischman 1989; Lamb et al. 1991). While incomplete, these data clearly suggest that human self-administration measures can reflect different and dissociable underlying processes from subjective self-reports of drug reward and more precise insights are likely to result from combining subjective rating assessments with self-administration that cannot be derived from studying subjective reports alone. For that reason, it is worth considering whether self-administration procedures may offer a preferred means of carrying out experiments in animals and humans to explore homologous processes across the species and to allow a more direct comparison of empirical outcomes.

Ethanol self-administration is well established in the rodent laboratory following the pioneering work of Hank Samson (Grant and Samson 1985; Roehrs and Samson 1981, 1982; Samson 1986; Tolliver et al. 1988). Although rodents frequently avoid taking ethanol, following (Samson 1986) they can be trained using a sucrose-fading technique (first trained to perform an instrumental response for a sucrose solution that is subsequently adulterated with increasing concentrations of ethanol, with parallel reductions, often to zero, in sucrose concentration). Such training has some features in common (face validity) with the typical pattern of human alcohol use that often begins by taking sweetened cocktails (alcopops) or cider, before progressing to more "adult" drinks that are unsweetened. Although rats have been most frequently used in this model, a limited number of studies have employed mice, and such models have been used to study genetic influences on alcohol self-administration, e.g. (Stephens et al. 2005a), and to test the efficacy of novel pharmacological approaches in treating alcohol abuse, e.g. (Middaugh et al. 2000).

However, despite the apparent similarity between self-administration in people and non-human animals, there are aspects of the procedures that may make them rather different. In animal studies, we are dealing with alcohol naïve subjects, with an inherent dislike of the taste of ethanol that requires extensive practice (i.e. the sucrose-fading technique) to overcome. In the human experimental laboratory, we inevitably train the instrumental response over a much shorter period of time (and often by instruction rather than self-discovery) in individuals who have already had considerable experience of ethanol and its effects (it would be considered unethical to expose ethanol-naïve subjects to the drug), not to mention long-term exposure to social contexts in which ethanol drinking may be accepted, encouraged or disapproved. It thus seems highly likely that the factors underlying self-administration of ethanol in human and non-human experimental animals are sufficiently different to suggest that the behaviors, though superficially similar (analogous), may not be homologous. For instance, human subjects have been reported to be willing to perform an instrumental response with a fixed ratio of >1000 (Bigelow and Liebson 1972; Zimmerman et al. 2011), while in outbred rats it is seldom the case that animals will perform at fixed ratios greater than about ten (though by using extended training techniques we have found it possible to achieve considerably higher ratios (up to 600) in outbred Lister rats (T.L. Ripley and D.N. Stephens, (unpublished)). Whether such apparent discrepancies reflect species-specific differences or differences in alcohol experience in laboratory humans and laboratory rats is unclear. Nevertheless, it then becomes an empirical question whether experimental manipulations that are effective in modulating ethanol self-administration in laboratory animals, are likely to do so in humans, as well. These issues are addressed in detail in an accompanying article (Zimmerman et al. 2011). Nevertheless, there may be sufficient in common between human and animal ethanol self-administration phenomena to allow self-administration procedures to be used in 'proof-of-concept' laboratory studies to evaluate drug reward value as a potential biomarker for examining possible treatment effects.

3.2 Conditioned Place Preference

One of the most commonly used tasks to study the "rewarding" effects of drugs (and hence their abuse potential) in rodent models is the place conditioning (or conditioned place preference, CPP) procedure, whereby a drug experience is repeatedly paired with exposure to a distinctive environment, while on separate occasions, a different environment is paired with a placebo/vehicle treatment; following such training, given a choice, non-drugged rats or mice should spend more time in the environment previously associated with treatment with a drug with abuse potential, an effect commonly interpreted as the animal having developed a "preference" for the drug-paired environment due to the rewarding

qualities of the drug being studied (see (Bardo and Bevins 2000; Cunningham et al. 2006; Stephens et al. 2010; Tzschentke 1998) for reviews).

Conditioned place preference has many advantages; it is relatively easy to perform and, in contrast to simple consumption studies, dose-effect curves are generally monotonic increasing, at least for ethanol studies in mice (Groblewski et al. 2008). However, perhaps because of its seemingly straightforward nature, there appears to be as many versions of place preference testing as there are laboratories using the task. Common differences include the number of environmental compartments (ranging from a single to three separate compartments), types of discriminative cues used (e.g., tactile, visual), visibility of the "drug environment" from the "non-drug environment", numbers of drug-environment pairings, duration of the drug-environment pairing and the use of unbiased versus biased designs. Although for many drugs of abuse, such as opiates and psychomotor stimulants, positive effects in the CPP procedure are sufficiently robust to withstand some variation in procedures, for others (e.g. ethanol) positive effects depend on judicious selection of the appropriate procedures, and/or may differ across species (Cunningham et al. 1993).

As we have pointed out elsewhere (Stephens et al. 2010), although seeming simple conceptually, the CPP procedure is difficult to interpret in terms of its underlying psychological processes, and its labelling with the heavily cognitive term "preference" is quite unfortunate. A general problem with the CPP procedure is that, with some praiseworthy exceptions, few researchers have attempted to explore systematically the psychological processes involved in drug- or non-drug place preference conditioning, and relatively little is known about how "preferences" for drug-paired stimuli develop and are maintained and expressed. As a consequence, it is mostly unclear how seemingly subtle methodological and procedural differences may or may not affect performance on this task, and differences across laboratories, such as those mentioned earlier, could reflect the way in which particular procedures bias the test to particular learning strategies. Thus, while CPP is commonly used to study the effects of both pharmacological manipulations, and gene associations with "reward" (e.g. Cunningham and Phillips 2003; Kliethermes et al. 2007), unless effort is spent on parsing putative component psychological (e.g., learning and memory) processes, the task may be insufficiently well understood to allow robust conclusions regarding homology with human measures. In rodents, for example, minor variations in the procedure are able to bias the test to assess processes as different as pavlovian approach (sign-tracking), the conditioned approach to positive incentives (Cunningham and Patel 2007; Mead et al. 2005), anxiolytic effects of the drug, or effects on learning. Without such knowledge, it becomes difficult to establish tests in human subjects that probe the same psychological processes as those in rodent CPP measures, and thus to achieve the aim of homology.

The complexity of the issue is illustrated by findings that the C57BL/6J mouse, in measures of consumption and choice, shows high consumption and preference for ethanol solutions relative to other strains, does not stand out in tests of ethanol CPP (Cunningham 1995). One possibility is that the development

of place "preference" in CPP reflects a balance of the aversive and rewarding effects of ethanol, so that variations in sensitivity to aversiveness interfere with assessment of reward (Cunningham and Henderson 2000; Cunningham et al. 2003).

While there are fewer examples available from the ethanol literature, a number of examples from other drugs further illustrate the high variability in outcomes using superficially similar CPP tests. For instance (Cunningham et al. 1999) reported that differences between C57BL/6J and DBA2/J mice in cocaine CPP were dependent on the length of the session. In another example, deletion of the *gria1* gene encoding GluR1 subunits of glutamatergic AMPA receptors has been reported both to abolish CPP for cocaine (Dong et al. 2004), and to have no effect (Mead et al. 2005), even though the Mead study covered the dose used in the Dong study. Finally, while (Valjent et al. 2006) reported deficits in cocaine-induced place preference conditioning (and the development of sensitization) in EGR1 (or Zif286) knockout mice using a three chamber CPP procedure (Li et al. 2006) found no deficits in CPP (or sensitization) in these mice when using a single chamber, but otherwise identical procedures. It is possible, of course, that these sorts of procedural or environmental differences between laboratories (including unspecified differences as simple as the rodent chow used by each laboratory) interact with the experimental manipulation, or with genotype to alter drug reward, perhaps by shifting the dose-effect curve for one strain, but not for the other. However, it is also possible that differences in the findings between two laboratories reflect subtleties in the place preference training and testing procedures that have nothing to do with reward.

In principle, it should be possible to establish a procedure similar to CPP in humans and a recent report (Childs and De Wit 2009) indeed confirms the development of place preference to amphetamine in humans. In this experiment, individuals experienced amphetamine in one of two environments, and were then asked to rate their liking for each of the environments using Likert-scales, as well as to express a preference for one of the environments. Liking was greater for the drug-associated room, and there was some evidence for a preference for the room that was paired with the drug, over the other non-drug-associated room. This is an interesting experiment, but it raises a number of questions that illustrate the problem of translation between animal and human models. In rodent CPP experiments, some researchers (Cunningham and Patel 2007; Mead et al. 2005) suggest that preference for a previously drug-paired environment reflects pavlovian approach–an automatic approach to cues associated with rewards and related to sign-tracking (sometimes, inappropriately called autoshaping). However, the human version of the task (Childs and De Wit 2009) rules out the possibility of pavlovian approach, and seems to reflect the drug-paired environment acquiring a cognitive label ("liked"). It will require further research before it becomes clear whether the psychological processes underlying this type of human place preference resemble those underlying the behavior in rodents tested in, an on-the-surface similar (analogous) task.

4 Models of Conditioning in Addiction

At present, most theories that describe the development and maintenance of addictive behavior agree on the important role of both Pavlovian and instrumental conditioning (e.g. Everitt and Robbins (2005); Franken (2003); Robinson and Berridge (1993)). Cues that are regularly associated with the consumption of a drug such as ethanol become conditioned stimuli (CS+) that are endowed with a broad range of emotional, cognitive and motivational functions. These include the ability to elicit conditioned emotional or physiological responses and subjective drug craving that are thought to modulate instrumental drug seeking behavior.

Conditioning models of drug addiction are widely used in animal research and a large number of studies, including from our groups, demonstrate that cues associated with drug experience can acquire incentive properties and trigger and maintain drug seeking (e.g. Everitt and Robbins 2005; O'Connor et al. 2010; Stewart et al. 1984). Related studies have also been conducted with human volunteers in the laboratory using arbitrary or neutral cues (e.g., the colour of the drinking vessel) associated with a reinforcer (e.g. an alcoholic drink) and show that such cues acquire salience and produce attentional, emotional and behavioral conditioned responses (e.g. Hogarth and Duka 2006). In the case of human subjects, one important condition necessary for the conditioned responses to occur is the expectancy of the reinforcer in the presence of the conditioned stimuli. As shown with smoking and alcohol stimuli, only if expectancies of the reinforcer have been explicitly activated, either by instruction (Field and Duka 2002) or by elaboration of the contingencies between the stimulus and reinforcement (Hogarth et al. 2005), do cues acquire the ability to alter attentional, emotional and behavioral processes. This finding may appear to differentiate cue reactivity in human subjects from procedurally similar phenomena in animals; however, the distinction may be more apparent than real as we have no way of knowing whether animals also develop an explicit (conscious?) awareness of the relationship existing between the reinforcer and the cue predicting it. Whatever the case, it remains a topic of debate, whether, and to what extent, awareness of the CS-US relationship is a necessary part of the process whereby CS-US associations are formed, as held by various expectancy theorists (Bolles 1972; Brandon et al. 2004; Dickinson 1989, 1997; Lovibond and Shanks 2002; Mackintosh 1997; Marlatt 1985). As noted, findings from contemporary human studies on conditioning support this view and suggest that the presence of awareness of the CS-US contingencies precedes the presence of conditioned responses (Hogarth and Duka 2006), but one alternative account could be that awareness and conditioning simply occur in parallel, and/or that the awareness aspect simply reflects the subject "reflecting" on and narrating about his/her own conditioned behavior.

The most commonly used approach to studying conditioning in the human laboratory involves measures of emotional reactivity to a conditioned stimulus, assessed by quantifying stimulus approach (attentional bias; see below), skin conductance responses and/or subjective feelings of pleasure following stimulus

presentation. Similarly, Pavlovian conditioning paradigms using emotional-reactivity measurements offer a tool for studying learning and memory processes involved in substance abuse and addiction (and more recently, for exploring underlying anatomical and neurochemical substrates), in the human laboratory.

There is a vast amount of behavioral evidence supporting the existence of attentional biases, which are closely correlated with Pavlovian approach responses (Buzsaki 1982) to drug-related stimuli in addiction-related disorders. These attentional biases become apparent during performance of attentional orienting tasks such as the MacLeod's dot-probe task, when tracking eye-movements, and/or using (secondary) task-interference procedures (e.g. the Stroop task). This type of attentional bias has been demonstrated for alcohol (Bauer and Cox 1998; Stetter et al. 1995; Townshend and Duka 2001), nicotine (Droungas et al. 1995; Glad and Adesso 1976; Herman 1974; Hogarth et al. 2003a; Niaura et al. 1994; Payne et al. 1991; Surawy et al. 1985), cocaine (Rosse et al. 1997) or opiate dependent subjects (Lubman et al. 2000). Importantly, the relevance of some of these attentional measures to the experimental medicine study of addiction is suggested by observations that a greater attentional bias to drug-related cues is predictive of poorer treatment outcome. This relationship has been found especially for attentional bias measured using the Stroop interference procedure using cues associated with alcohol (Cox et al. 2002), tobacco (Waters et al. 2003), heroin (Marissen et al. 2006) or cocaine (Carpenter et al. 2006). Thus, measures of attentional bias could offer themselves as surrogate measures of the effectiveness of potential therapeutic interventions. This appears to be a useful set of measures that can be reliably studied in human beings, and could offer a true homology in terms of psychological equivalence, hopefully based on a common neurobiology.

Although the methods described in the previous paragraph have little resemblance at a superficial level to the animal tests of CPP described in the previous section, in both cases, the underlying psychological processes are thought to depend (at least in large part) on approach behavior mediated by Pavlovian learning mechanisms. Of course, in the case of CPP, the approach may be to a particular context, whereas in the human studies discrete cues are used and the underlying neural networks are likely to diverge to an extent. For this reason, as outlined below, the use of animal tests employing simple, discrete cues may be more appropriate for establishing homologous approaches between animals and humans (see below).

Before discussing these approaches, it is worth noting that, as far as we are aware, no potential pharmacological treatments have been tested using the attentional cue-reactivity paradigm, although cognitive-behavioral treatment approaches aimed at altering drug user's attentional bias to drug cues have proven successful to some extent (Fadardi and Cox 2009; Field and Cox 2008). In addition, successful cognitive treatment based on 12 step individual and family psychotherapy, increases patients' insight into their inability to control their alcohol craving, and at the same time leads to avoidance of drug cues, i.e. attentional bias to drug-related cues is suppressed (Townshend and Duka 2007). Thus, attentional bias as a measure of reactivity to conditioned stimuli may offer a

useful biomarker for exploring the emotional, motivational and cognitive consequences of conditioning drug cues, as well as of brain substrates underlying these. A recent example of such an approach using functional magnetic resonance imaging (fMRI) by (McClernon et al. 2007) demonstrated how responsiveness to smoking-related cues is associated with increased BOLD responses within the amygdala (see also, e.g., Brody et al. 2002)—an area traditionally implicated in Pavlovian learning and emotional regulation, and this increase was reduced in abstinent smokers who had undergone cue-exposure (extinction) treatment (see below).

5 Studying Drug-Related Conditioning in Animals

5.1 Conditioned Reinforcement

Two well-described aspects of associative conditioning that may contribute to (different versions of) CPP in animal studies are conditioned or secondary reinforcement (CR) and Pavlovian approach. Conditioned reinforcement refers to the ability of environmental cues associated with the US reward to acquire reinforcing properties in their own right. Thus, it seems possible that approach to the environment associated with drug experience reflects "seeking" a conditioned reinforcer. Conditioned reinforcement is more conventionally, and more satisfactorily assessed using instrumental paradigms in which animals are shown to acquire a novel instrumental response to gain access to a discrete stimulus previously associated with the reward (Ferster and Skinner 1957; Robbins 1978). The brain regions involved in processing conditioned reinforcers are well explored and several studies have implicated the amygdala and orbitofrontal cortex (Holland and Gallagher 1999; Parkinson et al. 2000; Parkinson et al. 2001; Chudasama and Robbins 2003; Pears et al. 2003), as well as ventral striatum (Everitt et al. 1999) in this process. As conditioned reinforcement represents a relatively discrete and psychologically well-characterized behavior, with a well established neurobiology in rodents, it would seem to be an excellent candidate for translational work between animals and humans. Indeed, similar approaches have already been applied in human imaging studies, revealing the involvement of the same brain circuitry (Cox et al. 2005), so that there appears to be an emerging argument for homology between the rodent and the human phenomenon, and thus for a useful candidate for comparative studies between rodents and humans.

Nevertheless, a number of questions remain open. With one notable exception (Smith et al. 1977), there seem to be no published studies that have systematically varied the value of the unconditioned reward in order to quantify the effects on performance of the conditioned reinforcement task. This would seem to be an essential requirement if conditioned reinforcement were to be used to detect treatment effects on alcohol reward. Additionally, there is only limited evidence

that drug rewards support conditioned reinforcement (Di Ciano and Everitt 2004; Panlilio and Schindler 1997), so that while this approach may allow an assessment of reward sensitivity for conventional rewards, it is not yet clear whether the method is suitable to assay sensitivity to drug reward, though (Smith et al. 1977) reported the development of conditioned reinforcement using ethanol as the primary reinforcer.

5.2 Pavlovian Approach

As already noted above, a second psychological process likely to be invoked during some forms of CPP is Pavlovian approach learning that allows animals to spontaneously approach environmental stimuli that are predictive of reward. Such behavior is exploited in studies of sign-tracking, whereby animals engage with reward-predictive stimuli in a reinforcer- and species-specific manner (Brown and Jenkins 1968), even though the animal's behavior has no consequences for reinforcer availability (as no relation or contingency exists between the response and reinforcement probability). Thus, the 'preference' for an environment paired with drug reward might simply reflect approach to reward-predictive cues. Conditioned reward implies that the animal associates the positive incentive value to the reward-associated cues with performing a flexible or voluntary response; i.e. establishes a representation of goal-directedness of the response (Robbins 1978). In contrast, Pavlovian approach seems to be less flexible, and the shape of the behavior is determined by the nature of the cue and US (Gallagher et al. 1990). Importantly, while both conditioned reinforcement and Pavlovian approach rely on subjects establishing an association between the US and the CS, their expression appears to be mediated by different and experimentally dissociable neural systems (Parkinson et al. 2000). A recent study (Cunningham and Patel 2007) has demonstrated Pavlovian approach to a cue associated with alcohol administration in a procedure modified from a standard place conditioning procedure, perhaps suggesting that conditioned place preference is a variant of Pavlovian approach. Since sign-tracking has been demonstrated to occur in humans (Wilcove and Miller 1974), there seems to be an opportunity for cross-species concilience using this approach.

As mentioned above, a phenomenon related to Pavlovian approach is the orienting response to cues predictive of reward (Buzsaki 1982). In humans, this phenomenon has been exploited by studying the tendency of addicts to allocate attention to a stimulus associated with the drug over another stimulus that has no associative relations with the drug. These types of bias are readily measured by eye tracking techniques that quantify the focus of attention, or with cognitive interference tasks, during which allocation of attention to the emotional stimulus (reading a word with emotional meaning) takes up resources needed for current task demands (naming the colour of the ink in which the word is written). The relevance of this measure with respect to studying drug abuse is suggested by

observations that greater attentional bias to drug cues was associated with poorer treatment outcome and this relationship was demonstrated for alcohol (Cox et al. 2002), as well as other drugs including tobacco (Waters et al. 2003), heroin (Marissen et al. 2006) and cocaine (Carpenter et al. 2006).

As with conditioned reinforcement, a number of open questions need to be addressed before pavlovian approach might be exploited as an indirect measure of reward in both rodents and humans. Again, few studies appear to have been carried out relating magnitude of the primary reward to parameters of autoshaping (but see (Thomas et al. 1998)).

5.3 Pavlovian-Instrumental Transfer

Yet a third and neurobiologically dissociable, behavioral consequence of reward-associated cues is their ability to potentiate or energize ongoing instrumental responding even in the absence of any contingency between their presentation and the response (as is the case with conditioned reinforcement). Thus, cues previously associated with reward in Pavlovian training sessions are able to facilitate instrumental responding for that or other rewards when presented passively and independent of the subject's behavior, a phenomenon known as Pavlovian-instrumental transfer (PIT) (see (O'Connor et al. 2010)). Notably, depending on the particular Pavlovian training conditions, the cue may serve to facilitate responding for one particular reward (outcome-specific PIT), or a range of rewards (generalized form of PIT) and these two 'types' of PIT are again dissociable neurobiologically (e.g. (Corbit and Balleine 2005)). Although most work in the animal laboratory has used food rewards to establish the cue-reward association, two reports indicate that cues previously associated with ethanol delivery are capable of increasing instrumental responding for ethanol, consistent with ethanol-related cues facilitating ethanol-seeking behavior (Corbit and Janak 2007; Glasner et al. 2005). Nonetheless, PIT remains a phenomenon mostly studied and characterized using non-drug reinforcers and evidence for its role in drug-abuse and addiction is mostly theoretical.

Interestingly, the magnitude of PIT has been reported to be sensitive to behavioral sensitization procedures (repeated drug administration) induced following Pavlovian training (Wyvell and Berridge 2001), indicating that the extent to which drug-associated cues facilitate further drug seeking may be increased by drug-exposure history, though inducing ethanol dependence following pavlovian and instrumental training in itself does not further increase the facilitatory effects of ethanol-cues (Glasner et al. 2005). By contrast, in an experiment in which rats were chronically exposed to ethanol prior to pavlovian and instrumental training, the facilitatory effect of a food-paired cue on operant responding for food reward (PIT) was significantly impaired (Ripley et al. 2004). Thus, while ethanol reward clearly supports the development of PIT, ethanol dependence may impair the subsequent development of PIT using different (non-drug) rewards. Encouragingly, the PIT phenomenon is

readily reproduced in the human laboratory (Hogarth et al. 2007; Paredes-Olay et al. 2002), but, to our knowledge, no human studies have investigated PIT using ethanol-rewards.

5.4 Potential of Conditioning Measures as Biomarkers

The ability to establish test procedures in rodents and humans that are theoretically homologous provides an enormous advantage in moving between animal and human models. Although work remains to be done, all of the conditioning models outlined in this section appear to fulfil, or be capable of fulfilling, this requirement for construct validity of an animal model. However, although the importance of drug-related cues in triggering or strengthening drug seeking is well established, it is less clear which, to what extent, and under which conditions each of the specific processes outlined contribute to drug seeking or relapse in real life. Nevertheless, these approaches seem to offer a potential for validity not approached by more general animal models of addiction.

6 Subjective Experience

As noted above, evaluation of ethanol's effects in humans is heavily dependent upon subjective self-reports and direct measurements of subjective states in non-human animals are clearly tenuous. Then how is it possible to relate animal to human studies in this area?

Berridge and colleagues have proposed that some subjective experiences, namely hedonic reactions to tastes, can in fact be measured and inferred from facial expressions that are retained across mammalian species (Berridge 2000; Berridge and Grill 1983), perhaps offering a cross-species approach to study the subjective taste experiences. Although the taste-reactivity methodology is limited to orally administered substances, and thus of no straightforward use for studying subjective effects of drugs of abuse typically administered through other routes, it could offer a window for assessing alcohol-related subjective experience in rodents. In support of this notion, alcohol was found to produce similar orofacial responses in rats as sucrose/quinine mixtures (Kiefer et al. 1990). Moreover, Kiefer and colleagues (Bice and Kiefer 1990) have used the taste-reactivity procedure to compare subjective hedonic responses in alcohol preferring (P) and non-preferring (NP) rat strains finding that, after repeated experience with alcohol, P rats showed an increase in appetitive (hedonic) responses (and decreased aversive responses) to alcohol relative to NP rats. Thus, these initial findings suggest a possible methodology to obtain alcohol-related subjective reports that could be related to studies in human subjects.

The more classical means of studying "subjective" effects in animals is by the use of drug discrimination. In this methodology, the animal is required to use the discriminative stimulus provided by its experience of the drug's effect to make an appropriate response. Although the nature of the subjective experience of the animal remains unknown, it seems plausible that both human and non-human species could utilize the same subjective experience(s) of the drug in order to 'solve' the discrimination task (Kamien et al. 1993). Within the alcohol field, although there is a rich literature on discriminative stimulus effects in non-human animals (Grant 1999; Kostowski and Bienkowski 1999), there have been relatively few investigations into the nature of the discriminative stimulus in humans. Human volunteers are able to perform such discriminations based on ingestion of low doses of ethanol and discriminate based on a feeling of "light-headedness" engendered by the alcohol (Duka et al. 1999; Duka et al. 1998a). Notably, the alcohol discriminative stimulus generalizes to benzodiazepines which are also reported to generate feelings of "light-headedness" (Jackson et al. 2003). Such studies are consistent with rodent reports that an ethanol discriminative stimulus generalized to other sedative hypnotic agents such as benzodiazepines and barbiturates (Kostowski and Bienkowski 1999).

However, the human studies do not unequivocally relate alcohol's discriminative properties to its rewarding effects so that it is difficult to make the link between subjective reports, used in so many human studies, with discriminative performance. Nevertheless, subjective ratings of the stimulant effects of alcohol predict the amount of alcohol subsequently consumed (Duka et al. 1998b). Thus, although subjective reports assay something that is several levels of neural processing away from behavioral measures, correlations between these domains can sometimes be established. Nevertheless, there is only a limited basis for the use of animal drug discrimination experiments as a non-human surrogate for investigating human subjective reports.

7 Alcohol and Impulsivity

A recent focus of addiction researchers in the animal and human lab has been on decision-making processes and the role of frontostriatal dysfunction in addiction, following on from the early proposal of Jentsch and Taylor (1999). This focus stems, in part, from neurocognitive evidence that addicts often show persistent performance deficits on decision-making tasks reminiscent of focal damage to prefrontal (PFC), ventromedial PFC (Verdejo-Garcia and Bechara 2009) or orbitofrontal (Rogers et al. 1999) cortex. Within the alcohol field, a number of reports demonstrate a relationship between alcohol misuse and impulsivity (see Dick et al. 2010 for a recent review). Important in this discussion is the recognition that the term impulsivity is used in human studies to describe a number of rather different behaviors, including rash acts arising from "sensation seeking, risk-taking, novelty-seeking, boldness, adventuresomeness, boredom susceptibility, unreliability, and unorderliness" (Depue and

Collins 1999). A useful attempt has been made by (Whiteside and Lynam 2001) to map these complex concepts into five traits that are only weakly related (see (Dick et al. 2010) for more extensive discussion and references). These traits encompass "positive" and "negative urgency" (acting rashly when experiencing, respectively, positive and negative mood), "lack of planning" (acting without forethought), "lack of perseverance" (failure to tolerate boredom, or to remain focussed in the face of distraction) and "sensation seeking", the tendency to seek novel or thrilling stimulation). These traits are derived essentially from personality inventories, so that a major question for the present article is to what extent these traits can be operationalized in humans, and then in animal models homologous with the human experimental procedures.

(Dick et al. 2010) point to the difficulty in reconciling performance measures in laboratory tasks with personality traits, as the former reflect specific cognitive processes under particular experimental conditions, whereas the latter are stable traits that are likely to be independent of particular tests, and thus broader than specific cognitive functions. De Wit and colleagues (Reynolds et al. 2006a) provide a more general analysis relating personality inventories and behavioral measures of impulsivity, and suggest that self-report measures assessed using such inventories are generally unrelated to task-based measures. Nevertheless, a number of experimenters have recently related specific laboratory measures to personality traits. Among these is the suggestion that "urgency" may be reflected in ability to inhibit prepotent responses (Bechara and Van der Linden 2005), whereas lack of perseverance may relate to resistance to proactive interference, and these suggestions have found some empirical support (Gay et al. 2008; McCarthy et al. 2001).

De Wit and colleagues (Reynolds et al. 2006a) also suggested that the laboratory tasks they used could be allocated to two categories, the first which they labeled "impulsive disinhibition" (that included a 'Stop-task' and a 'Go/No-Go task'), and a second (that included 'Delay-Discounting' and the 'Balloon Assessment of Risk Task' (BART)) which they labeled "impulsive decision making". This is an important distinction that has also been described by others (Lane et al. 2003), and may map onto the categories "impulsive action" and "impulsive choice" used by others in the field.

Within the animal literature, Evenden (Evenden 1999) has made a similar point, that impulsivity is a term with several meanings. This recognition has played an important role in helping to define the theoretical bases of animal tasks purporting to measure "impulsivity". Animal tasks can essentially be divided into those that measure the inability to withhold a response ("impulsive disinhibition"), or intolerance to delays in reward or perseveration of a non-rewarded response ("impulsive decision making"). Although several tasks fall within these descriptors, two tasks have become increasingly popular; the 5-choice serial-reaction time task (5-CSRTT) (Robbins 2002) that measures response (i.e. motor) inhibition, and the "delay-discounting" tasks (e.g. Richards et al. 1997). Although not encompassing all types of impulsivity, these tasks may give a reasonable assessment of the two basic concepts of "impulsive action" and "impulsive choice".

However, the relationship of performance in the two tasks to alcohol abuse is complex. Alcohol given acutely has little effect in human volunteers in a delay-discounting task (Dougherty et al. 2008; Richards et al. 1999), while intoxicating amounts tend to induce discounting of delayed rewards at lower rates (Ortner et al. 2003). De Wit and colleagues (Reynolds et al. 2006b) suggest that failures to find effects may reflect particular aspects of the task used, including the use of hypothetical question-based measures, and, indeed, using a task in which real delays were experienced (experiential discounting task, EDT) report that acute doses of alcohol induce aversion to delayed reward. This latter task is much more closely related to the kind of delay-discounting used in animal models and one might expect similar effects of acute ethanol in rodent delay-discounting tasks, though we are unaware of such studies having been performed.

In contrast to the mixed effects of acute alcohol on task performance, chronic use in heavy drinkers or alcoholics appears to produce intolerance to delays (e.g. (Field et al. 2007; Mitchell et al. 2005)). Thus, increased sensitivity to delay may contribute to loss of control over drinking behavior, while ethanol itself appears not to further affect this kind of impulsivity. Findings consistent with these conclusions have also been reported in rodent models. For example, rat strains selected for high alcohol drinking are more sensitive to delay than rats selected for low ethanol consumption (Wilhelm and Mitchell 2008). And, similarly, alcohol-naïve outbred mice selected for high alcohol drinking are more impulsive in a delay-discounting task (Oberlin and Grahame 2009), suggesting that sensitivity to delay may be a predisposing factor in alcohol use.

In the case of the 5-CSRTT, alcohol given acutely did not increase numbers of premature (impulsive) responses in mice in the standard, over-trained form of 5-CSRTT (Oliver et al. 2009), and a similar effect was observed in rats (Bizarro et al. 2003). However, when premature responding was provoked during probe trials by increasing the inter-trial interval Oliver et al. (2009) found 1 g/kg ethanol increased impulsivity. This result may suggest that actions that are performed habitually can be insensitive to effects of ethanol, while in non-habitual situations, in which the subject is required to adapt its behavior and respond accordingly to novel requirements, impulsive-like behavioral performance is sensitive to the effects of alcohol.

We are not aware of published data using the 5-CSRTT in rodent strains bred for alcohol preference, but (Patel et al. 2006) reported that C57BL/6 mice (sub-strain not specified) were less impulsive than DBA/2 mice in a version of the task in which premature responding was not punished. This observation is unexpected as mice of the C57BL/6J strains are known to consume alcohol more readily than DBA/2 mice and, in our own unpublished experiments using C57BL/6J mice, we find them to be more impulsive than DBA2/J mice during 5-CSRTT probe sessions using extended intertrial intervals. These comparisons point to influences of subtle variations in task requirements on performance that need careful attention when comparing human and animal methods.

With regard to the increase in impulsive responding after ethanol treatment, our results are in agreement with other reports using different paradigms of impulsivity

in rats, including the delay-of-reinforcement paradigm, where ethanol increased impulsive behavior (Evenden and Ryan 1999; Olmstead et al. 2006; Poulos et al. 1998; Tomie et al. 1998) suggesting that ethanol given acutely increases both impulsive choice and impulsive action. Similarly, in human studies, in measures of response inhibition, when the subject is required to withhold an already initiated response (e.g. Stop-signal tasks), ethanol seems to increase impulsivity in moderate drinkers and in college students (Dougherty et al. 1999, 2000; Mulvihill et al. 1997).

Thus, in a number of laboratory tasks designed to tease apart specific aspects of impulsive behavior, there appears to be good consistency between animal and human laboratory tasks, both in terms of measures that predict high alcohol consumption, and in the acute effects of alcohol. These are valuable findings as, on the one hand, they allow confident use of the animal tests to predict effects of treatment in humans, while on the other, they allow the use of experimental manipulations such as CNS lesions, to probe the circuitries that contribute to alcohol's effects, with a reasonable confidence that such observations have relevance for human alcohol abuse. As with the conditioning measures outlined above, however, it is less clear as yet as to which aspects of impulsivity, are causal in determining alcohol abuse, and which behavioral measures are best to complete the link between the animal model and the clinical reality.

8 Withdrawal from Alcohol

So far we have dealt with problems associated with assessing behaviors in animals and humans that might allow us to proceed with confidence from animal findings to human experimentation, and to the clinic. However, when it comes to studying the effects of alcohol (or other addictive drugs) there is an equally important discussion to be had regarding treatment-related parameters and what constitutes equivalent "treatment" regimens in animal and human experiments. Such a discussion is particularly important when considering the long-term consequences of alcohol abuse.

A feature of alcoholism is the long-term consumption of large amounts of the drug. Such a pattern leads typically to tolerance to its effects, development of dependence revealed as withdrawal signs and symptoms on cessation of use, and toxic effects arising from alcohol itself, its metabolite acetaldehyde, or secondary to alcohol-induced organ damage especially hepatic disease. Furthermore, withdrawal from alcohol is itself associated with neurobiological changes that underlie some of the symptoms of withdrawal but that may also have an impact on long-term brain function after the withdrawal symptoms themselves subside. In recent years there has been a growing interest in the effect of repeated episodes of withdrawal from ethanol (detoxification) on central nervous system functioning in both human and non-human animal studies. Many of these important clinical features of alcoholism are not at all readily reproduced in animal models.

A number of ways are used by health and regulatory authorities to define heavy drinking, so that, for instance, in many countries it is illegal to drive with blood alcohol levels exceeding 50 or 80 mg/dL. UK governmental recommendations point to consumption in excess of 4 units of alcohol per day for a man, or 3 units for a woman as being likely to compromise health. Common definitions of binge drinking refer to blood alcohol levels of 80 mg/dL on a given occasion (Lange and Voas 2000; NIAAA 2004). Some alcoholic patients are likely to reach and maintain blood alcohol levels much in excess of these levels for protracted periods of time.

In the human laboratory, it would frequently be considered unethical to give sufficient alcohol to approach these kinds of blood alcohol level on even a single occasion, while to model persistent alcohol abuse is clearly out of the question. Even when studying the effects of an acute dose of alcohol, which aspects need to be modeled?—The amount? The maximum blood alcohol level achieved? The time to reach peak blood concentrations? The rapidity of offset? And if these questions are impossible for the human researcher, what aspects of these patterns of drinking does the animal researcher interested in longer-term effects of alcohol abuse need to consider? Clearly, a major issue arises from the simple fact that rodents and humans differ markedly in their ability to metabolize alcohol, so that attempts at equalizing consumption (say on a body weight basis) simply do not allow parallels in blood alcohol concentrations over a 24 h time period. And how should the animal researcher integrate features of human alcohol abuse such as drunkenness and blackouts, into his models? At an operational level, these problems are clearly insoluble. Nevertheless, and surprisingly, a number of methods of administering alcohol to rodents to achieve high blood alcohol levels (say, greater than 100 mg/dL) have been effective in inducing behavior and neurobiological consequences that appear at some level to parallel the consequences of alcohol abuse in human. Perhaps the task of modeling alcohol abuse in animals can be solved not on the "input" side, but on the "output" side. In that case, modeling the dynamics of blood and brain alcohol levels may be less important than modeling the consequences, both behavioral and neurobiological, of alcohol abuse. A number of attempts at establishing such parallels between behavioral outcomes have been documented in the literature, particularly in the context of the consequences of binge drinking.

9 Binge Drinking and Withdrawal

Binge drinking was originally used to describe the periods of excessive drinking among alcoholics followed by periods of abstinence. Over the last 20 years binge drinking has been used more frequently to describe the excessive drinking of alcohol, often with harmful consequences, increasingly common among adolescents and college students (Midanik et al. 1996; Wechsler et al. 1994). In particular, there is a fear that a binge drinking pattern of alcohol consumption may cause brain damage in both humans and animals (Hunt 1993).

There have been several definitions of binge drinking. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) has approved the following definition: 'A 'binge' is a pattern of drinking alcohol that brings BAC to about 0.08 gram-percent or above. For the typical adult, this pattern corresponds to consuming five or more drinks (male), or four or more drinks (female), in about 2 h' (NIAAA 2004). This definition ignores differences in body size, or natural tolerance, or the development of tolerance, and for that reason, in our own studies of binge drinking, we have used a more behavioral approach based on the alcohol use questionnaire (Mehrabian and Russell 1978), which incorporates speed of drinking, and the behavioral measures, 'numbers of times being drunk in the last 6 months' (with drunkenness defined as loss of coordination, nausea and/or the inability to speak clearly, or blackout) and the percentage of times getting drunk when drinking (Townshend and Duka 2002). Although differences in definition of binge drinking may give rise to some confusion both in the scientific literature and among the general public, it is likely that the multiple definitions tap into closely related phenomena.

Binge drinking, in addition, is characterized by repeated bouts of drinking leading to high levels of alcohol in the brain followed by periods in which brain alcohol levels return to zero. We have proposed that binge drinking may lead to brain damage and resultant cognitive dysfunction, which may be similar to the neurotoxicity induced by repeated withdrawals from alcohol in dependent animals and humans (Crews et al. 2001; Duka et al. 2004; Duka et al. 2003; Stephens and Duka 2008; Stephens et al. 2005b; Veatch and Gonzalez 1999).

There are several rodent models of binge drinking although most of them do not model the "free choice" aspect of human drinking. Intermittent alcohol vapor administration over several days with concentrations of alcohol in blood reaching as much as 250 mg/dL leads to several features typical of dependence on humans. These include altered sleep patterns (Criado et al. 2008; Ehlers and Criado 2010) and kindling of withdrawal seizures (Becker et al. 1997a, b). Recent reports suggest that such treatments also lead to reduced amplitude of Event Related Potentials also suggesting impairments in cognitive functions (Criado and Ehlers 2010). However, the extent to which these changes in rodents map on to homologous changes in human binge drinkers has not been tested, so that, while these observations may be of interest in their own right, we do not know the extent to which they provide an adequate model of the consequences of human binge drinking.

A number of models use acute i.p. or oral gavage administration of ethanol to achieve high blood alcohol levels. One such model provides a 4-day ethanol "binge" of 3 g/kg administered via oral gavage every 8 h leads to cumulative blood alcohol levels of 250 mg/dL. Such treatments induce brain tissue shrinkage with increased lateral ventricular volumes (Zahr et al. 2009). Similarly, "binge" ethanol exposure in adult rats (induced by daily i.p. injections) causes necrotic neurodegeneration after as little as 2 days of exposure (Obernier et al. 2002). As far as we are aware, no such extreme events are observed in human binge drinking, so that although these reports may point to potential consequences of

extreme alcohol abuse in humans, one must question the validity of such approaches as models of binge drinking.

Since human binge drinking is often emphasized (probably incorrectly) as an adolescent phenomenon, many researchers are interested in modeling the effects of alcohol during developmental stages. Thus, Crews and colleagues (Crews et al. 2000) have found that young adolescent rats show a different pattern of brain damage after binge ethanol administration from that found in adult rats, so that damage was sustained to the associated frontal cortical olfactory regions in the adolescent, but not adult rats. Subsequent animal studies have confirmed such neurotoxic effects of excessive alcohol drinking in the adolescent brain. Studies with human adolescents and university students, which examined the effects of heavy binge drinking, have suggested alcohol-related brain structural (De Bellis et al. 2000, 2005; Medina et al. 2007; Nagel et al. 2005) and functional (Hartley et al. 2004; Tapert et al. 2004; Townshend and Duka 2005) abnormalities. In humans, the prefrontal lobe continues to mature into the early twenties (Casey et al. 2000; Gogtay et al. 2004), leading to the suggestion that this late developing area may therefore be especially sensitive to heavy alcohol use during adolescence. These are interesting and potentially important findings that appear to map rodent data on to parallel human observations. However, there is some way to go before we can regard such observations as homologous. Development of the rodent brain takes place with a time course quite different from that of the human brain, so that “adolescence” in the rat may reflect different stages in brain development from human “adolescence”. Giving two things the same name does not make them the same thing! Moreover, there are features of human prefrontal cortex that have no homologous structure in the rodent. Clearly, great care must be taken before we can map rodent findings on to human binge drinking.

The preceding paragraphs concern themselves with the consequences of alcohol exposure on withdrawal, and on neurotoxic effects. Similar models of alcohol exposure have also been used to study the importance of alcohol exposure on motivation for alcohol. For instance, rats exposed to repeated cycles of intoxication and withdrawal using the ethanol vapor method to achieve blood levels substantially greater than 200 mg/dL, increase their voluntary intake of ethanol (Rimondini et al. 2002; Sommer et al. 2008), and show greater motivation for ethanol (Schulteis et al. 1996).

Spanagel (Spanagel and Holter 1999) developed a model which allows animals to choose among four different drinks with regard to alcohol concentrations (0, 5, 10 and 20% v/v). The bottles are introduced into the animal’s cage and become part of their everyday life (very much like alcohol for young adults). With experience, the average daily alcohol intake reaches approximately 6.5 g/kg. Following different periods of voluntary alcohol drinking, the animals can be deprived for one or more days and, following the deprivation period, alcohol can be introduced again. Animals will then increase their drinking, which becomes compulsive (alcohol deprivation effect). The same group has also shown an increased reactivity to stressful stimuli during the periods of abstinence (Sanchis-Segura and Spanagel 2006). This model for studying the long-term consequences

of drinking and the mechanisms of relapse thus has considerable face validity, but, like all the other methods outlined in this section, has limited theoretical basis. For that reason, it is difficult to know whether potentially important neurobiological findings, such as changes in NMDA receptors associated with the alcohol deprivation effect (Vengeliene et al. 2005), are likely to carry over to the human condition.

Another model that gives animals free access to alcohol, but hardly free choice, is the model used by Stephens et al. (2001). In this model animals are trained to accept alcohol, using free access to a 5% alcohol nutritionally complete liquid diet as their sole food source. Restricted periods of alcohol administration (8 days alcohol diet–3 days non alcohol diet) allow control over alcohol drinking and patterns of drinking. Following this procedure, we have demonstrated that 24 days of alcohol interspersed by two periods of 3 days of alcohol deprivation leads to dependence as measured by increased sensitivity to convulsions 7 days after the last withdrawal (Stephens et al. 2001). Animals drink alcohol at an equivalent dose of 15–20 g/kg/day and blood alcohol reaches a level of 100 mg/dL (Ripley et al. 2003). One advantage of this model is that by using, in addition to a control group that does not take any alcohol, a group which is exposed to 24 days of alcohol intake but without the 3-day alcohol-free periods, it is possible to assess the effects not only of alcohol intake but also of the repeated withdrawal experience. The animals with the three withdrawals from alcohol show a greater sensitivity to repeated pentylentetrazol-induced proconvulsant activity compared to animals which experienced only one withdrawal (Ripley et al. 2002) suggesting that they undergo a greater degree of physical dependency, and they also demonstrate increased motivation for ethanol (Brown et al. 1998).

While this model is not intrinsically "better" than the other consumption or exposure models outlined above (and indeed its "face value" could be argued to be considerably less than the Spanagel model), we have made some effort to map the behavioral changes induced by this "binge" model on to homologous behavioral changes in binge drinking humans, and alcoholic patients. Thus, for instance, rats exposed to repeated episodes of withdrawal show impairments in aversive conditioning (Stephens et al. 2001) that are also observed in a homologous task in young human binge drinkers (Stephens and Duka 2008; Stephens et al. 2005b). We have observed such parallel changes in a number of laboratory measures in which we have been able to develop closely homologous behavioral tests in rats and humans (see (Stephens and Duka 2008)). These similarities in the behavioral outcomes of binge drinking and the animal model gives us some confidence that the model reproduces some of the effects of human binge drinking, and may therefore allow us to draw conclusions using the model that might have relevance to the human condition beyond the particular tests we have carried out. Thus, for instance, that behavioral effects seen in binge drinkers can be induced in the rodent model through alcohol exposure and withdrawal, may suggest that similar abnormalities found in binge drinkers may reflect the consequences of drinking, rather than predating it (for a review see (Stephens and Duka 2008)).

Of course, the opportunities for establishing homologous findings in the rodent model and the human are limited, and function best in carefully controlled laboratory experiments such as outlined above. The problem then arises of the relevance of the carefully controlled human laboratory behavior to behavior of alcohol abusers in the real world. In some cases, it may be possible to make direct links between human laboratory studies and real-world behaviors. For instance, there is a good theoretical basis for thinking that fear conditioning, as studied most easily in the laboratory, may underlie aspects of normal and abnormal anxiety. In such cases, we can, via the human laboratory studies, extrapolate our rodent findings more broadly to the clinical condition.

However, in many cases, the link between the human laboratory findings and the real world is more tenuous, so that the human laboratory findings may be better viewed as analogous to the real world situation. In such cases, we will nevertheless feel more confident in relating our rodent observations to real-world alcohol abuse, whereas in the absence of the human laboratory step, such relationships would be no more than appeals to “face value”.

There are many situations in which it is impossible to achieve the ideal “rodent model—human laboratory model—human clinical experience” triad, especially where it would be unethical or unfeasible to conduct the human laboratory test. For instance, we have found that in the repeated withdrawal model, rats that have undergone repeated withdrawals subsequent to a conditioning experiment, subsequently generalize their fear learning to cues not previously paired with the aversive stimulus (Stephens et al. 2005b); to carry out the analogous experiment in humans would require fear conditioning to be established prior to experience of binge drinking, followed by test. This is clearly impractical, as well as unethical. Nevertheless, we have found analogous inappropriate generalization in binge drinkers in an aversive conditioning paradigm (Stephens et al. 2005b). The close parallels we have seen in homologous tasks using the same rodent model allow us to draw parallels also in the inappropriate generalization findings. Furthermore, it allows us to speculate that overgeneralization of fear that we have observed in quite different tasks in alcoholic patients and binge drinkers (Stephens and Duka 2008) may reflect a related phenomenon, a speculation we would have been reluctant to make in the absence of the intermediate human lab data.

10 Adolescent Drinking

With the recent increase in binge drinking and incidence of drunkenness with its consequences (e.g., violent behavior) among adolescents, research interests have been diverted to studying binge drinking in adolescent animals and humans. The challenge of this approach is not only to model the binge drinking procedure, so as to mimic the drinking behavior seen in adolescents, but also to choose the appropriate age in animals that reflects the developmental cerebral stage in humans.

Adolescence is a time of rapid brain reorganization through "pruning" and myelination. During this period brain structure may be especially sensitive to alcohol effects. Teenagers with alcohol use disorders show reduced hippocampal (De Bellis et al. 2000; Nagel et al. 2005) and prefrontal cortex volume (De Bellis et al. 2005; Medina et al. 2008). fMRI studies indicate altered responses to verbal (Tapert et al. 2004) or visual (Crego et al. 2010) working memory tasks as well as a verbal encoding tasks (Schweinsburg et al. 2010) in teenagers with alcohol use disorders.

To what extent can such observations be modeled in rodents? Clearly, verbal memory lies outside the possibilities of animal studies, but inasmuch as processes that underlie verbal working memory reflect similar processes as those underlying visual working memory, then parallel tests might be developed. Equally, if it is established that a rodent model of adolescent alcohol abuse leads to similar consequences for, say, prefrontal cortex function as is seen in human adolescents, then it would make sense to use behavioral tasks requiring intact function of those areas in rodents to study consequences of ethanol abuse, especially if tasks can be developed that have close parallels in humans, and are known to be impaired in human adolescent alcohol abuse, irrespective of whether there is a close parallel between the details of alcohol treatments (amounts or timing) in rodents and humans.

In other words, it may be more important to establish homology in the behavioral outputs of animal models than in achieving close similarities between human patterns of drinking, and alcohol treatment in the model (i.e. on the "input" side of the model).

11 Conclusion

The current article argues that a weakness in much laboratory research on alcohol is the uncritical attribution of "face validity" to behavioral analyses of alcohol's effects. This is a problem for much preclinical psychopharmacology research in most areas of mental health. We argue that a more rigorous (though less ambitious) approach might be to eschew attempts at developing animal models of the general human condition, in favor of establishing limited, but closely homologous behavioral tests in animals and humans that allow a better understanding of the psychological processes impacted by alcohol abuse, and governing alcohol-seeking behavior, as well as more reliable (though limited) predictions from the animal test to the homologous human test. In parallel, it might be possible to use such limited human tests as "biomarkers", or "intermediate behavioral phenotypes" for the particular condition of interest (Duka et al. 2010). Such tests would not claim to model the entire human condition, but would be concerned at identifying fundamental aspects of the underlying behavioral pathology that give rise, untreated, to the wider disorder. We suggest several aspects of alcohol-related behavior for which parallel tests might be developed (or have already been

developed) for rodents and humans though in the present article we have not extensively explored potential biomarkers (but see Duka et al. (2010)).

We have recognized that modeling patterns of human alcohol abuse in animals is fraught with difficulties, so that making predictions for human alcohol abuse from observations of the consequences of a particular treatment regimen for a particular behavior in animals is hazardous. Given the major differences in physiology between the human and rodent, attempts to arrive at better models of alcohol abuse by manipulating patterns of consumption may be predestined for failure. Nevertheless, we are struck by the ability of some animal models of alcohol abuse to mimic behavioral changes in human alcoholic patients, or binge drinkers in behavioral tests designed to be closely homologous between the human and the rodent. Such similarities in the behavioral output of human alcohol abuse, and the rodent model might indicate that the existing rodent models are able to mimic important aspects of the human abuse pattern.

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A Translational Approach to Novel Medication Development for Protracted Abstinence

Barbara J. Mason and Amanda E. Higley

Abstract Alcohol dependence is a chronic relapsing disorder. Despite significant strides in the development of efficacious behavioral and pharmacological treatments for alcohol dependence, relapse rates remain very high. In this chapter, we review validated animal and human laboratory models for assessing risk of relapse in alcohol dependence and neurobiological treatment targets derived from such models. We suggest a translational approach to evaluate potential pharmacological treatments, using existing medications to validate and refine research paradigms across clinical and pre-clinical domains, with the aim of providing an accelerated framework for medications development in alcohol dependence. Lastly, empirical findings from proof-of-concept human laboratory studies are reviewed as we discuss the importance of selecting human laboratory models with predictive validity for the mechanism of action of the drug undergoing evaluation for efficacy in alcohol dependence.

Keywords Human laboratory models · Relapse · Protracted abstinence · Medication development · Translational models · Cue reactivity · Craving

B. J. Mason (✉) · A. E. Higley
Committee on the Neurobiology of Addictive Disorders,
The Scripps Research Institute, La Jolla, CA 92037, USA
e-mail: bmason@scripps.edu

B. J. Mason
The Pearson Center for Alcoholism and Addiction Research,
Laboratory of Clinical Psychopharmacology, The Scripps Research Institute,
10550 North Torrey Pines Road, TPC-5La Jolla, CA 92037, USA

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Contents

1 Introduction 648
2 Neurobiology of Alcohol Dependence 648
3 Clinical Manifestation of Protracted Abstinence 651
4 Preclinical Models 652
5 Clinical Paradigms 652
5.1 Cue Reactivity 653
5.2 Affective Priming 654
5.3 Stress-Induced Craving 654
5.4 Alcohol Self-Administration 656
6 Implications for Medication Development 657
7 Summary and Concluding Remarks 660
References 661

1 Introduction

Alcohol use disorders, which include both alcohol abuse and dependence, comprise one of the most prevalent categories of substance use disorders, affecting more than two billion people worldwide. The diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV; American Psychiatric Association 2000), characterizes alcohol dependence as a maladaptive pattern of drinking leading to clinically significant impairment, as manifested by a compulsion to drink, a lack of control over the amount of alcohol consumed, and continued drinking despite realization of the associated problems. Although significant strides have been made in the development of efficacious behavioral and pharmacologic treatments for alcohol dependence, relapse rates remain very high. Relapse, or the return to alcohol abuse following periods of abstinence, is one of the principle characteristics of dependence on alcohol. A recent Betty Ford consensus panel identified complete abstinence as the most reliable way for formerly dependent individuals to avoid relapse and its associated problems (Betty Ford Institute 2007). Given that one of the most challenging aspects of recovering from alcohol dependence is maintaining abstinence, understanding the factors underlying relapse susceptibility is of particular importance. Research indicates that alcohol-associated cues, negative affective states, and stress are common triggers to relapse, or the return to drinking (Higley et al. 2011; Mason et al. 2008; Sinha et al. 2009).

2 Neurobiology of Alcohol Dependence

Neurobiological approaches suggest that alcohol dependence develops in a process of homeostatic adaptation to chronic high doses of alcohol that increases set point for reward (Koob 1998; Koob and Le Moal 1997). Several neurochemical systems

and brain regions are engaged in the development of alcohol dependence (for review see, Koob and Le Moal 1997). Such neuroadaptations may result in the emergence of negative affective states and stress responses upon discontinuation of alcohol use that are motivational to relapse in dependent people. The process is thought to involve loss of function of several neurotransmitter systems including dopamine, serotonin and opioid systems, and recruitment of brain stress systems including CRF, norepinephrine, and dynorphin (Koob and Le Moal 2001; Koob and Volkow 2010).

Short-term alcohol consumption tilts the balance in favor of inhibitory influences as well as decreases the function of excitatory neurotransmitters. With long term, chronic alcohol exposure, the brain compensates by upregulating excitatory mechanisms while concurrently decreasing inhibitory neurotransmission to help restore equilibrium. Persistent upregulation of excitatory mechanisms may ultimately result in neuroadaptive changes that lead to alcohol dependence and tolerance (Littleton 1995). Upon removal of alcohol, this excitatory state remains unopposed, resulting in hyperexcitability and dysfunction that is characteristic of both acute and protracted alcohol withdrawal (Swift 1999). Moreover, these neurotransmitter systems modulate internal states associated with positive and negative affect (Koob and Le Moal 1997) which are implicated in clinical vulnerability to relapse in protracted abstinence (Addolorato et al. 2005; Marlatt and George 1984). Protracted abstinence involves a state of heightened relapse vulnerability driven by dysregulation in stress and reward systems in the CNS extending long beyond acute alcohol withdrawal (Koob 2006; Martinotti et al. 2008b). Clinically, this state involves symptoms of craving, sleep disturbances, and negative affective states all of which have been identified as risk factors for relapse (Breese et al. 2005; Fox et al. 2008; Mason and Lehert 2010).

Alcohol is a powerful activator of the stress response. Chronic alcohol use is associated with several abnormalities of the stress response which could have important implications for our understanding of the neurobiology of dependence and relapse. Specifically, alcohol-dependent individuals show a blunted cortisol and ACTH response to acute stressors (Berman et al. 1990; Wand and Dobs 1991), an effect that remains for up to 12 weeks after cessation of drinking (Bernardy et al. 1996; Ehrenreich et al. 1997; Errico et al. 1993; Lovallo et al. 2000), and these attenuated HPA responses are associated with alcohol relapse (Junghanns et al. 2003). Recent research suggests that neural systems mediating behavioral stress responses may offer useful targets for pharmacotherapy of alcoholism.

Stress relief during protracted abstinence is thought to be a major motivation for excessive alcohol consumption. The physiologic mechanism of stress relief following alcohol consumption is thought to occur mainly in the extended amygdala outside the hypothalamic-pituitary-adrenal (HPA) system. However, the HPA axis may contribute to the dysregulation of the extended amygdala stress system. Acute alcohol administration has been shown to enhance levels of HPA axis hormones in humans and animal models (Koob and Le Moal 1997). As dependence on alcohol develops, HPA axis activity appears to become dysregulated, and over time, chronic exposure to alcohol may actually decrease the responsivity of the HPA

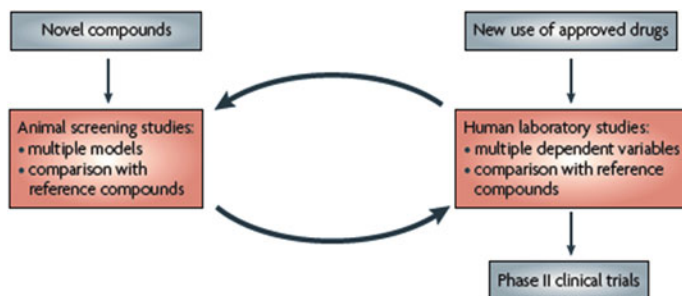

Fig. 1 The 'Rosetta Stone approach' to drug development. A crucial aspect of the proposed Rosetta Stone approach is the dynamic feedback from animal models and clinical data which can be used to identify treatments for drug addiction that are likely to succeed in clinical trials and to facilitate further development of animal and human models. These data may ultimately provide a rational basis for combination therapies such that multiple components of the addiction cycle can be treated by a given pharmacological strategy. From Koob et al. (2009) figure reprinted with permission from Nature Publishing Group

axis to external stimuli and sensitize extrahypothalamic brain stress CRF systems in the amygdala. Together these neuroadaptations impair the subject's ability to cope with relapse inducing stressors (Junghanns et al. 2003; Koob and Volkow 2010; Le et al. 2000; Zorrilla et al. 2001).

These alcohol-induced neurobiological changes represent possible molecular targets for pharmacotherapies of alcoholism. Pharmacotherapies for alcoholism may either modulate or block the rewarding effects of alcohol or stabilize neurobiological systems dysregulated by chronic alcohol intake, which help to facilitate abstinence or greatly reduce alcohol consumption. Consistent with these concepts, evidence is available that pharmacological treatments can support abstinence or decrease the number of heavy drinking days. In the United States there are three medications approved for the treatment of alcohol dependence; disulfiram, naltrexone, and acamprosate. Recent efforts to develop new medications include a focus on neural response to risk factors for relapse drinking during protracted abstinence. A key rationale for the study of modulators of the brain emotional systems in drug dependence treatment is that medications that normalize the dysregulation or balance of the reward and stress systems may protect against relapse.

There is a substantial need for discovering innovative ways to provide more information on the neurobiology of alcohol dependence as well as to discover more effective pharmacotherapies for alcohol dependence. A combination of validated animal models for addiction, neurobiological targets derived from such models, and translation to and from the clinical domain provides a dynamic framework that can be used to identify possible treatments for alcohol dependence and symptoms of protracted withdrawal that are likely to succeed in clinical trials and to facilitate further development of animal and human models (see Fig. 1).

This translational approach may accelerate medication development whereby validated animal and human laboratory models of protracted abstinence may be used to screen potential pharmacotherapies for alcoholism and drug dependence disorders.

3 Clinical Manifestation of Protracted Abstinence

Withdrawal symptoms that emerge after cessation of drinking are characteristic of alcohol dependence and are strongly correlated with relapse to compulsive drinking. In addition to the acute physical symptoms associated with detoxification, the protracted withdrawal syndrome has emotional and affective symptoms such as anxiety, irritability, and depressed mood (Bokstrom et al. 1989, 1991; Roelofs 1985; Roelofs and Dikkenberg 1987). Symptoms of anxiety, depression, sleep disturbances, and elevated stress response may not be restricted to the acute alcohol withdrawal stage, but have been reported to last weeks, months, and even years after cessation of drinking, in a protracted withdrawal syndrome (Alling et al. 1982; Janiri et al. 2005; Voltaire-Carlsson et al. 1996; Watanabe et al. 2001). Clinical reports of withdrawal symptoms extending beyond detoxification from alcohol have been described in different studies. Segal et al. (1970) tested patients 2 months after their last alcohol intake and reported that 45% manifested a persistent syndrome characterized by mood liability, irritability, apathy, insomnia, impaired concentration, and cognitive function; whereas Begleiter and Porjesz (1979) found persisting states of anxiety, insomnia, and complaints of pain in patients evaluated 3–6 weeks after last alcohol use. Fatigue and tension have been reported to persist up to 5 weeks post-withdrawal (Alling et al. 1982; Martinotti et al. 2008a). Anxiety has been shown to persist up to 9 months and, in 20–25% of alcoholics, anxiety and depression have been shown to persist up to 2 years post-withdrawal (Roelofs 1985). These post-acute withdrawal symptoms of protracted abstinence tend to be affective in nature, subacute, and often precede relapses (Annis et al. 1998; Hershon 1977).

Protracted abstinence is a state that involves symptoms of anxiety, irritability, hostility, dysphoria, insomnia, fatigue, and craving and is hypothesized to be driven by dysregulation in stress and reward systems in the central nervous system (CNS) that persist long past acute withdrawal from alcohol (De Soto et al. 1985; De Witte et al. 2003; Koob 2003; Martinotti et al. 2008b; Mason et al. 2009; Mossberg et al. 1985; Roberto et al. 2006; Schuckit et al. 1990). Clinical literature has indicated that protracted abstinence symptoms of craving, negative affect, and sleep disturbances are strongly correlated to relapse to compulsive drinking (Annis et al. 1998; Breese et al. 2005; Cloninger 1987; De Soto et al. 1985; Fox et al. 2008; Hershon 1977; Mason and Lehert 2010). For example, Hershon (1977) showed that 94% of the alcohol-dependent subjects experienced anxiety following cessation of drinking, and 82% of these patients reported an alleviation of this symptom after alcohol use.

4 Preclinical Models

Animal models of addiction have outstanding face validity (for example, intravenous self-administration) and recapitulate aspects of the human condition. They also have substantial construct validity (for example, deregulated stress responsivity during drug withdrawal). Also relevant to understanding addiction are the animal paradigms modeling components of the motivational aspects of withdrawal and the negative reinforcing effects of dependence, which can be used to explore how the nervous system is involved in motivation to seek drug and adaption to drug use. These include anxiety-like responses, conditioned place aversion, elevated reward thresholds and withdrawal-induced increases in drug self-administration. Animal models of craving include the conditioned rewarding effects of drugs of abuse, measures of the conditioned aversive effects of withdrawal, and signs and symptoms of protracted abstinence (Koob 2006; Sanchis-Segura and Spanagel 2006). Further vulnerability for relapse following abstinence can be explored utilizing drug-, cue-, and stress-induced reinstatement paradigms.

Consistent with the clinical literature, laboratory animal studies of alcohol dependence have demonstrated both behavioral and physiological changes that extend beyond the acute withdrawal phase and contribute to a vulnerability to relapse. Of note, several studies have shown that protracted withdrawal following alcohol intoxication is accompanied by upregulation or increased function of several brain neurotransmitter systems, such as CRF, norepinephrine, and dynorphin, which are known to mediate anxiogenic-like responses (Koob 2008). Data from established animal models of anxiety suggest that a major consequence of withdrawal from chronic alcohol exposure is increased anxiety as modeled by behavioral changes in the elevated plus-maze (Baldwin et al. 1989, 1991; Rassnick et al. 1993), acoustic startle (Macey et al. 1996), and social interaction tests (Overstreet et al. 2002) that persist for up to 4 weeks following chronic alcohol exposure (Rasmussen et al. 2001). Laboratory animals examined in animal models of alcoholism display distinct behavioral changes that are characteristic of an enhanced responsiveness to stressful stimuli (Baldwin et al. 1991; Holter et al. 1998; Moller et al. 1997; Rassnick et al. 1993) and heightened vulnerability to relapse (Liu and Weiss 2002). Although no animal model fully reproduces addiction in humans, such models do permit investigation of elements of the addiction process and provide the theoretical framework and neurobiological targets for medication development which is an important goal of neurobiological research in alcoholism and addiction.

5 Clinical Paradigms

Human laboratory studies provide a powerful means of exploring pharmacological treatment targets for each stage of the addiction cycle prior to conducting expensive, double-blind, placebo-controlled clinical trials. Moreover, human

laboratory studies can potentially identify appropriate efficacy measures for clinical trials of prospective pharmacotherapies for each stage of the addiction cycle, investigate real-world constructs such as vulnerability to addiction, impulsivity, craving, and resistance to relapse, and translate basic science models of relapse to the clinical context.

5.1 Cue Reactivity

Craving is often a target of pharmacological- and cognitive-behavioral therapeutic interventions for alcohol dependence based on evidence suggesting that it may increase the likelihood of relapse among alcoholics following treatment (Monti et al. 1993; Rohsenow et al. 1994). Human laboratory models are particularly well suited to investigate mechanisms of craving given the immediacy of effects obtained under relatively well-controlled conditions (Litt and Cooney 1999). Human models of cue reactivity assess craving by using a laboratory setting to recreate risk conditions for relapse similar to those experienced by alcoholics in their natural environment (Litt and Cooney 1999). The typical methodology employed in alcohol cue-induced reactivity studies has been to expose alcohol-dependent subjects to the sight and smell of their preferred alcohol-containing beverage versus a nonalcoholic control beverage (e.g. bottled water) as an analog of a high-risk situation. Increased physiological reactivity (i.e., elevated heart rate, blood pressure, galvanic skin response) and urge to drink have been found when alcoholics are exposed to the sight and smell of their preferred alcoholic beverage in a laboratory setting (Carter and Tiffany 1999; Cooney et al. 1997; Monti et al. 1987; Sayette et al. 1994). This response set is known as cue reactivity, and has been found to be more intense in alcoholics than nonalcoholics (Kaplan et al. 1983) and to increase in relation to severity of alcohol dependence (Glautier and Drummond 1994). Moreover, a relationship has been found between the measure of cue reactivity and subsequent drinking which lends support to the predictive validity of cue reactivity as an analog for clinical outcomes (Cooney et al. 1997; Monti et al. 1999; Rohsenow et al. 1994).

In a proof-of-concept study our laboratory used a paradigm of affective priming followed by exposure to alcohol cues to investigate the efficacy of gabapentin to treat symptoms of protracted abstinence. Gabapentin, an anti-convulsant drug with GABA modulating actions, is hypothesized to normalize dysregulation in brain stress systems caused by alcohol dependence. Treatment with gabapentin significantly decreased craving in the presence of alcoholic beverage cues, as well as positive affect-induced craving, and arousal induced by both positive and negative affective stimuli and improved several measures of sleep quality (Mason et al. 2009). Similarly, acamprosate, the prototypic neuromodulating drug for treating protracted abstinence in alcohol dependence, attenuated reactivity to alcohol cues significantly more than placebo across subjective measures of craving for alcohol and also significantly improved naturalistic measures of sleep quality and craving

(Mason et al. 2010). These findings provide robust support for utilizing the human laboratory model of cue reactivity to assess novel drugs for efficacy in protracted abstinence.

5.2 Affective Priming

Theories of protracted abstinence and cue reactivity have predicted an important role for emotional state as a risk factor for relapse based on evidence from both naturalistic, (Marlatt 1985; Vuchinich et al. 1996) and laboratory studies (Cooney et al. 1997; Litt et al. 1990; Mason et al. 2008, 2009). The presence of an alcoholic beverage is an important contributor to relapse (Marlatt 1985), but exposure to alcohol alone does not reflect the emotional factors often associated with relapse. Prior work has shown that subjective and physiological reactivity to the sight and smell of alcohol (i.e., exteroceptive cues) is enhanced by induction of affective states. Induction of negative affective states in the cue exposure laboratory has been associated with increased reactivity (Cooney et al. 1991; Litt et al. 1990; Rohsenow et al. 1994; Rubonis et al. 1994). Positive affective states have also been induced in the laboratory and were associated with significantly greater urges to relapse then neutral affective states (Mason et al. 2008; Tiffany 1999). Niaura (2000) provided a theoretical basis for the role of positive affect in a feedback loop in which expectation of reward and intent to use may spiral upwards, leading to relapse. This may be the case for some abstinent alcoholics who report their most relapse-prone mood state is positive, e.g., during exciting sporting events (Marlatt 1985).

5.3 Stress-Induced Craving

Converging lines of evidence indicate that stress increases risk of addictive behaviors. Early life stress and childhood maltreatment, chronic cumulative adversity, major life trauma, and negative emotionality are associated with increasing levels of drug use and abuse (Brady and Sonne 1999; Brown et al. 2002; Nemeroff 1996; Uhart and Wand 2009) and have been associated with relapse and vulnerability to relapse (Koob and Kreek 2007; Marlatt 1990). Chronic alcohol use is associated with alteration in the brain stress and reward responses, including changes in the activities of both the hypothalamic-pituitary-adrenal (HPA) axis and dopaminergic activity; moreover, such changes are associated with increases in alcohol craving (Adinoff et al. 1991, 2003, 2005a, b; Cleck and Blendy 2008; Gilman and Hommer 2008; Heinz et al. 2004, 2005; Koob and Kreek 2007; Koob et al. 2004; Martinez et al. 2007; Volkow 2004) and shorter time to relapse in early abstinent individuals (Higley et al. 2011; Junghanns et al. 2003, 2005).

In human laboratory studies exposure to stress and negative affective cues have been shown to increase alcohol craving and stress-related physiological arousal (Breese et al. 2005; Cooney et al. 1997; Litt et al. 2000; Marlatt 1990; Mason et al. 2009; Sinha 2009; Sinha et al. 2000). Moreover, several early studies showed that acute stress, (induced by insoluble arithmetic or interpersonal evaluations) increased alcohol consumption in social drinkers (de Wit et al. 2003; Higgins and Marlatt 1975; Hull and Young 1983; Miller et al. 1974). Using personalized guided imagery to induce stress, Sinha et al. (2009) found increased craving for alcohol accompanied by a blunted cortisol response in nontreatment seeking alcohol-dependent adults. Similarly, we designed a human laboratory paradigm of stress induction using individualized stress scripts (Higley et al. 2011). Alcohol-dependent subjects, enrolled in a 12 week clinical trial for alcohol dependence, participated in a 1 h stress script development session with a trained clinician during the first visit of study participation. During the script development session (modified from Pitman et al. 1987) subjects were asked to identify a recent highly stressful event from their own lives (rated by the subject as ≥8 on a 10-point Likert-type scale for stressfulness). A structured stimulus response interview was used to elicit specific details of the stressful event. Subjects were then asked to select from a "menu" those subjective visceral and muscular reactions that he/she remembered as having accompanied the experience (Pitman et al. 1987). Immediately following the script development session the clinician developed a 1.5 min script that portrayed the stressful experience in the second person, present tense, and incorporated 5 different visceral and muscular reactions or as many as the subject selected, whichever was less. The script was then audio recorded for later use in the laboratory session. Examples of commonly reported stressful situations included breakup with a significant other, a verbal argument with a significant other or family member, or employment-related stress, such as being fired or laid off from work. Stressful situations involving alcohol use or repercussions from alcohol use were not allowed so as not to confound stress-induced craving with alcohol cue-related craving.

The human laboratory session was conducted at week 2 of treatment. Standardized measures and self report questionnaires were collected to provide pre-manipulation baseline ratings of drinking, craving, and mood. After completing all baseline assessments, subjects were escorted to a comfortable chair located in a windowless, sound-attenuated testing room adjacent to the control room and separated by a large one-way mirror. Subjects were familiarized with the laboratory procedures during a neutral practice trial. Pre-manipulation salivary cortisol and subjective VAS alcohol craving ratings were obtained as markers of baseline cortisol and craving response. At 2:00 pm subjects were provided with headphones and a digital audio recorder and instructed to listen to the audio recording for the entire duration and try to remember and imagine the event as if it were happening at that time. Immediately following the script presentation, salivary cortisol samples and subjective VAS alcohol craving ratings were obtained. Stress-induced craving was assessed using four individual 20-point visual analog scale (VAS) items adapted from the ACQ (Singleton et al. 1994) items. Each VAS

item endpoint was anchored with a 0 on the left indicating no craving, and a 20 on the right indicating severe craving. The items represented strength of craving ("How strong is your craving to drink alcohol"), impulse ("It would be hard to turn down a drink right now"), control ("If I could drink alcohol now, I would drink it"), and relief drinking ("Having a drink would make things just perfect"). Total stress-induced craving was calculated by summing the four individual items. Finally, subjects were asked to indicate on a 10 point VAS the degree to which the script evoked a stress response as a validation check of the laboratory-based manipulation of stress exposure.

Results indicated that greater stress-induced craving was associated with a blunted salivary cortisol response, significantly shorter time to alcohol relapse, higher mean drinks per week, fewer percent days abstinent, and lower rates of complete abstinence over the study duration (all p's < 0.05; Higley et al. 2011). Conversely, no demographic or baseline variables were significant predictors of any treatment outcome variable. These results support the use of stress-induced craving as a predictor of alcohol relapse propensity. Furthermore, treatments that address high stress levels and the associated high levels of alcohol craving are likely to improve treatment outcome in alcohol dependence.

5.4 Alcohol Self-Administration

Self-administration of alcohol in the laboratory is a useful tool to study effects of potential pharmacotherapies for the binge/intoxication phase of addiction. In one design, subjects are presented with a tray of alcoholic drinks and are invited to consume as many of them as they like, or to receive monetary compensation for each drink they reject. Thus, the total number of drinks, or blood alcohol concentration achieved are the outcome measures. This type of experiment is presumably influenced by several distinct factors that may not be affected by drug, including sensitivity, and tolerance to alcohol, maintenance or loss of control, taste preferences, personality traits such as impulsivity, and the kinetics of gastrointestinal absorption. A further problem with oral alcohol administration is that even after adjusting dosages for total body weight (thus minimizing the effects of sex and body morphology) and performing the ingestion with identical experimental procedures, the maximum observed BAC and the time of its occurrence after oral ingestion vary about threefold between subjects (Ramchandani and O'Connor 2006). This variability complicates the interpretation of self-administration experiments as subjects ingesting the same sequence of drinks will differ substantially in their brain alcohol exposure. The impact of the many influential factors that contribute to alcohol self-administration cannot be easily dissected; nonetheless, it is a measure with high face validity in respect to the binge/intoxication phase, as the dependent variable comprises the target behavior of drinking.

Infusing alcohol intravenously can overcome many of the problems of alcohol self-administration. Researchers have recently developed a physiologically based

pharmacokinetic (PBPK) model of alcohol administration and elimination (Plawecki et al. 2007, 2008). In this paradigm, the arterial (rather than venous) BAC is controlled which is a better representation of brain alcohol exposure and can be reliably measured using breath samples (Lindberg et al. 2007). The PBPK model calculates an individualized infusion protocol maintaining arterial BAC within 5 mg% of the target concentration. The same principle was used to achieve rapid linear changes of arterial BAC with minimal experimental variability across subjects (O'Connor and Lang 2007). A computer assisted self-infusion of ethanol (CASE) model has recently been developed which employs the PBPK model to achieve an identical increment in arterial BAC each time a subject chooses to self infuse, rather than administering a fixed dose with drinking (Zimmermann et al. 2008). An important facet of the CASE method is that subjects do not know much alcohol they have infused nor how often they are supposed to push the "drink" button. Therefore, their decisions for or against taking another "drink" are based solely on the pharmacological alcohol effects they perceive. Thus, the effects of a potential pharmacotherapy on the binge/intoxication phase of dependence may be assessed with fewer confounding factors. From a learning theory point of view, another advantage of the CASE paradigm is that the contingency between the behavior (pushing the button to receive a "drink") and its consequences (feeling a change in alcohol effect) is closer than with oral administration for two reasons. First, each button press results in the exactly the same amount of arterial BAC increase in every subject at anytime throughout the experiment. Second, all these aBAC increments follow exactly the same kinetics (i.e., a linear increase over a preset period of time) thus; increments are achieved with much more reliability than would be possible with drinking. Therefore, CASE enables human subjects to gain more direct control over their brain alcohol exposure than with oral self-administration and makes other implications like individual preferences for specific alcoholic beverages, brands, tastes, and/or smells irrelevant.

6 Implications for Medication Development

Human laboratory models permit sensitive and systematic evaluations of medication efficacy on affective states and drinking urges, alone and in combination, that have been reliably associated with drinking relapse (Mason et al. 2008). Human laboratory paradigms can predict treatment outcome (Cooney et al. 1997; Higley et al. 2011) and have been validated in some cases using medications that successfully treat alcoholism (see Table 1). Ongoing research exploring neurobiological pathways involved in alcohol dependence creates opportunities for the development of novel medications that may prove to be safe and effective in the treatment of alcohol dependence. To date emphasis has been placed on treatments to block the reinforcing effects of alcohol. The first two medications approved for alcohol dependence, disulfiram, and naltrexone, targeted reduction in pathological alcohol use by reducing the rewarding value of alcohol. Both drugs, however, have

Table 1 Drug efficacy in human laboratory models of cue reactivity and alcohol self-administration relative to randomized, placebo-controlled clinical trials

Drug		Cue reactivity		Self-administration		Phase II/III
Acamprosate	+	Hammarberg et al. (2009); Ooteman et al. (2007); Weinstein et al. (2003)	–	NM	+	Baltieri and de Andrade (2004); Barrias et al. (1997); Besson et al. (1998); Geerlings et al. (1997); Gual and Lehert (2001); Kiefer et al. (2003); Ladewig et al. (1993); Lhuintre et al. (1985); Mason et al. (2006); Niederhofer and Staffen (2003); Paille et al. (1995); Pelc et al. (1992, 1997); Poldrugo (1997); Sass et al. (1996); Tempesta et al. (2000); Whitworth et al. (1996)
					–	Anton et al. (2006); Chick et al. (2000b); Morley et al. (2006); Namkoong et al. (2003); Roussaux et al. (1996)
Gabapentin	+	Mason et al. (2009)	–	Myrick et al. (2007); Bisaga and Evans (2006)	+	Brower et al. (2008); Furieri and Nakamura-Palacios (2007)
Ondansetron	+	Myrick et al. (2008)*		NM	+	Johnson et al. (2000, 2002); Sellers et al. (1994)
Topiramate	–	Miranda et al. (2008)		NM	+	Baltieri et al. (2008); Johnson et al. (2003, 2004, 2007)
Naltrexone	+	Monti et al. (1999); Myrick et al. (2008)*; McGeary et al. (2006); Ooteman et al. (2007); Palfai et al. (1999); Rohsenow et al. (2000)	+	Anton et al. (2004a); Davidson et al. (1996, 1999); Drobes et al. (2003); McCaul et al. (2000); O'Malley et al. (2002); Ray and Hutchison (2007)		Anton et al. (1999, 2006); Balldin et al. (2003); Guardia et al. (2002); Heinala et al. (2001); Kiefer et al. (2003); Kranzler et al. (1998, 2003, 2004, 2009); Latt et al. (2002); Morris et al. (2001); O'Malley et al. (1992); Petrakis et al. (2005); Volpicelli et al. (1992)

(continued)

Table 1 (continued)

Drug	Cue reactivity	Self-administration	Phase II/III
	− McGeary et al. (2006); Modesto-Lowe et al. (1997); Palfai et al. (1999)	− Davidson et al. (1999); de Wit et al. (1999); Plafai et al. (1999)	− Baltieri et al. (2008); Chick et al. (2000a); Davidson et al. (2004); Gastpar et al. (2002); Huang et al. (2005); Killeen et al. (2004); Krystal et al. (2001); Martinotti et al. (2010); Morley et al. (2006); Oslin et al. (1997, 2008); Volpicelli et al. (1997)
Nalmefene	NM	+ Drobes et al. (2003)	+ Mason et al. (1994, 1999)
			− Anton et al. (2004b)
Rimonabant	NM	− George et al. (2010)	− Soyka et al. (2008)

(+) Significant effect of drug; (−) No significant effect of drug; *NM* Not Measured
* Naltrexone and Ondansetron were given in combination

been characterized by problems with noncompliance (O'Farrell et al. 1995; Volpicelli et al. 1997) and a return to pathological drinking when the drug is discontinued (Anton et al. 2001). An alternative, more clinically relevant emphasis may be on the negative emotional states associated with protracted abstinence (Baker et al. 2004; Koob and Le Moal 2008; Marlatt 1979). Medications that normalize the dysregulated motivational systems specifically associated with alcohol dependence may serve to prolong abstinence and have ancillary benefits, e.g., on sleep, that serve to enhance compliance (Staner et al. 2006).

When using human laboratory models to screen potential pharmacotherapies for addiction, it is critical to choose the model appropriate for the mechanism of action of the drug under study to avoid false negative findings. For example, clinical trials have consistently shown that acamprosate and naltrexone are both active agents for the treatment of alcohol dependence. However, each drug seems to work via unique mechanisms of action. Acamprosate modulates central glutamatergic receptor function and may exert its therapeutic action by decreasing an alcoholic's 'need' to drink (Heilig and Egli 2006; Mason 2005) by normalizing protracted dysregulation in brain systems caused by chronic alcohol use and withdrawal. In contrast, naltrexone exerts its effect by blockade of opioid receptors, which are involved in alcohol's rewarding effects on the brain (Galloway et al. 2005). As a result, a patient drinking alcohol while on naltrexone is hypothesized to experience less reinforcing euphoria resulting in less consumption which is appropriately measured in the alcohol self-administration paradigm of the human laboratory models. Conversely, acamprosate may be most appropriately studied in the cue reactivity paradigms and not in those relying on alcohol administration, as alcohol administration would negate the condition of protracted abstinence neuromodulatory drugs are hypothesized to treat.

7 Summary and Concluding Remarks

Human laboratory models of risk factors for relapse in alcohol dependence may be used as efficient screens of potential, novel medications for an alcohol indication. If such a model demonstrates predictive validity it may inform the risk/benefit assessment of conducting the lengthy and costly clinical trials mandated for the regulatory approval of novel pharmacotherapies for alcohol dependence. Available pharmacotherapies for alcohol dependence have limited efficacy, possibly as a function of their action at limited neuropharmacological targets for such a multifaceted disease. Applying known treatments for addiction to validate existing animal and human laboratory models may accelerate the translation of new targets to pharmacotherapies for addiction. Table 1 shows the efficacy of human laboratory models to predict success in clinical trials. Models applying cue reactivity procedures correctly predicted positive outcomes for clinical trials of neuromodulatory drugs such as acamprosate and gabapentin. Conversely, alcohol administration procedures incorrectly predicted negative outcomes for clinical trials with

these same drugs. Alcohol administration procedures show their greatest success in assessing drugs hypothesized to reduce the rewarding effects of drinking, e.g., naltrexone or nalmefene. Cue reactivity models show superior predictive validity for neuromodulatory drugs hypothesized to treat the negative effects of NOT drinking in alcohol-dependent individuals, e.g., the alcohol craving, mood, and sleep disturbances associated with heightened risk for drinking relapse in protracted abstinence.

Applying known treatments such as acamprosate and naltrexone to existing animal and human laboratory models serves as a back check on a model's predictive validity. Table 1 shows the efficacy of selected human laboratory models for predicting the success of known and novel drugs in Phase II and III clinical trials for alcohol dependence. The selection of animal and human laboratory models that are appropriate for a drug's mechanism of action may accelerate the translation of new targets to pharmacotherapies for addiction.

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New Approaches to Addiction Treatment Based on Learning and Memory

Falk Kiefer and Christina Dinter

Abstract Preclinical studies suggest that physiological learning processes are similar to changes observed in addicts at the molecular, neuronal, and structural levels. Based on the importance of classical and instrumental conditioning in the development and maintenance of addictive disorders, many have suggested cue-exposure-based extinction training of conditioned, drug-related responses as a potential new treatment of addiction. It may also be possible to facilitate this extinction training with pharmacological compounds that strengthen memory consolidation during cue exposure. Another potential therapeutic intervention would be based on the so-called reconsolidation theory. According to this hypothesis, already-consolidated memories return to a labile state when reactivated, allowing them to undergo another phase of consolidation–reconsolidation, which can be pharmacologically manipulated. These approaches suggest that the extinction of drug-related memories may represent a viable treatment strategy in the future treatment of addiction.

Keywords Addiction · Dependence · Reconsolidation · LTP · Reward · Extinction · Cue exposure

List of Abbreviations

BLA	Basolateral amygdala
DCS	D-cycloserine

F. Kiefer · C. Dinter
Department of Addictive Behavior and Addiction Medicine,
Central Institute of Mental Health, University of Heidelberg,
Square J5, 68159 Mannheim, Germany

F. Kiefer (✉)
Department of Addiction Medicine, Central Institute for Mental Health,
Square J5, 68159 Mannheim, Germany
e-mail: falk.kiefer@zi-mannheim.de

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LTD Long time depression
LTP Long-term potentiation
NAc Nucleus accumbens
NMDA *N*-methyl-D-aspartate
PFC Prefrontal cortex
VTA Ventral tegmental area

Contents

1 Introduction: Learning and Memory in Addiction 672
2 Dopamine and Glutamate: Key Molecules 673
3 The Role of Long-Term Potentiation and Neural Plasticity in Addiction 675
4 Novel Treatment Approaches: Cue-Exposure Therapy 676
5 Novel Treatment Approaches: Forced Extinction 677
6 The Theory of Reconsolidation in Addiction 678
7 Conclusions 680
References 681

1 Introduction: Learning and Memory in Addiction

Drug and alcohol addiction are characterized by cravings, difficulties in controlling intake, development of tolerance and dependence, as well as a dramatic decline in social functioning not related to drug or alcohol intake. Addiction is a chronic-relapsing disease: even after long periods of abstinence, the risk of relapse—often precipitated by drug-associated cues—remains high (Mann et al. 2005). Several studies have suggested that learning processes that continue even once someone stops their intake (*addiction memory*) play an especially large role in the maintenance of addictive behaviour (Boening 2001; Everitt et al. 2001; Hyman 2005; von der Goltz and Kiefer 2009).

Preclinical studies suggest that physiological learning processes are similar to the changes observed in addicts at the molecular, neuronal and structural levels. The brain is not a static organ, but is restructured by *neuroplastic* processes which are shaped by both stimulation and experience (Cooke and Bliss 2006; Bliss and Lomo 1973). Learning includes not only the initial perception, processing and storage of information but also the subsequent ability to orient future behaviour with regard to this new information (Kandel et al. 2000). *Reward learning* plays a particularly crucial role in the development of addiction. In reward learning, consequences of hedonic behaviours initiate learning processes, in particular those that positively value the cues associated with rewards (Hyman et al. 2006).

Considering the persistence of addictive disorders, it is reasonable to assume a specific and time-stable memory system. The so-called long-term memory can be broadly divided into the consciously accessible, declarative memory (or explicit memory) and the unconscious, non-declarative or *implicit memory*. Since both habit learning and simple conditioning forms are assigned to the implicit memory (as well as to the procedural memory), addiction memory is made up mainly of non-declarative contents. It has been suggested that the neuronal plasticity induced by chronic substance misuse leads to the formation of an implicit memory.

Addictive behaviour is learned predominantly in phylogenetically old brain systems involving the *mesolimbic reward system*. Projections from the ventral tegmental area (VTA) of the midbrain reach into the ventral striatum, and from this relay station signals are passed on into the prefrontal cortex (PFC). Additionally, there are various reciprocal projections between the VTA and the hippocampus—a crucial brain area for explicit learning and memory—which link motivational and reinforcing signals together in the encoding of new cues (Lisman and Grace 2005). The core and shell areas of the Nucleus Accumbens (NAc) (the main part of the ventral striatum) are distinguished from each other both anatomically and functionally. While the shell region of the NAc processes the influence of primary (unconditioned) reinforcers, the core area reacts to classically conditioned cues (Ito et al. 2004). The basolateral amygdala (BLA) also conditions associations between motivational and emotionally relevant events, and helps mediate behavioural responses to previously conditioned cues (Everitt and Robbins 2005). Imaging studies have shown the prefrontal cortex to be central to attention, decision making and executive functions in the pathogenesis of addictive behaviour (Goldstein and Volkow 2002; Kalivas and Volkow 2005). Preclinical and imagine studies have shown neuroplastic and functional changes in all of the aforementioned brain regions over the course of the development and maintenance of addictive disorders.

Humans and animals rapidly *learn cues and contexts* that help them predict the availability of addictive drugs. Once learned, these cues and contexts initiate drug seeking, craving and relapse both in animal models and in clinical studies in humans. These observations have led to the hypothesis that addiction represents the pathological usurpation of neural processes that normally serve reward-related learning (Wise 1987). A substantial body of research suggests that several types of such neuroadaptation occur, including synapse-specific adaptations of the type thought to underlie specific, long-term associative memory (Hyman et al. 2006).

2 Dopamine and Glutamate: Key Molecules

Dopamine plays a central role in reward-related learning (Wise 2004). Directly or indirectly, all addictive drugs (including alcohol) increase levels of synaptic dopamine within the nucleus accumbens (Di Chiara and Imperato 1988). In a series of experiments, Schultz et al. (1997) investigated the circumstances under

which midbrain dopamine neurons fire in the context of reward. They found phasic bursts of dopamine transmission to be related to a reward-predicting, conditioned stimulus (i.e. a light or a tone), and not to the reward itself. The firing rate of dopamine neurons pauses following unreinforced exposure to stimuli associated previously with reward. They hypothesized that these phasic bursts and breaks together encode a prediction-error signal, and that they represent the neuronal basis for conditioning and extinction of drug–cue associations. In their *incentive sensitization theory of addiction* Robinson and Berridge (1993, 1998, 2000, 2001) make the assumption that the neuroadaptations that follow from repeated drug consumption render mesolimbic circuits hypersensitive to drugs and drug-associated stimuli. They therefore hypothesize that drug-associated stimuli both capture one's attention and are motivationally salient ("incentive salience"). They emphasize that the sensitization of reward pathways not only relates to the emotional evaluation of the drug (liking), but more importantly relates to the incentive salience of drug-related cues (wanting). The important point that Robinson and Berridge emphasize is that a history of drug learning may lead to implicit drug memories that are not open to conscious remembering, and that drug-related cues may therefore trigger automatic, drug-related responses the person is not necessarily aware of (see also McCusker 2001).

Insights into the role of dopamine in processing reward prediction helped make learning the model on which addictive disorders are understood (Wise 2004). The analogy between learning and addiction begins with the fact that all addictive substances share the ability to increase dopamine concentrations in the NAc via projections from the VTA (Di Chiara and Imperato 1988). Computer models of reward-associated learning have been used to extend results from preclinical studies on the role of dopamine, in order to better represent the neuronal basis for the conditioning of drug–cue associations (Montague et al. 2004). These computer models are based on the hypothesis that individuals tend to direct their behaviour at increasing their likelihood of obtaining future rewards. The reward system encodes its prediction of reward either as "better than expected," with a phasic increase (positive prediction error), or "worse than expected," with a phasic decrease (negative prediction error) of dopaminergic transmission (Schultz et al. 1997). Due to the pharmacologically induced increase in dopaminergic transmission that addictive drugs cause, the signal "better than expected" is generated independently of the subjective hedonic effect of the substance. Although it remains controversial whether it is the dopaminergic transmission itself that initiates learning processes, or whether these processes are caused indirectly with the help of other neuronal systems, we do know that dopamine promotes memory consolidation, and that a blockage of dopaminergic transmission has the opposite effect (Dalley et al. 2005; Lisman and Grace 2005).

Dopamine is intimately involved in the processes that condition and reinforce both the acquisition of preferences and the shift of attention from one object to another. This involvement is responsible for the fact that substance-induced plasticity of glutamatergic neurons in the PFC, including its projections to the NAc, is associated with compulsive drug consumption as well as chronic relapsing

behaviour in the advanced stages of addiction. These two effects can be mediated by reducing the capacity of the PFC to respond to biological rewards, or to inhibit its ability to provide executive control over drug seeking (Kalivas and Volkow 2005). Several preclinical studies have shown that addictive behaviour in cocaine addicts (after chronic consumption of the drug) is mediated by increased glutamatergic transmission between the PFC and the core region of the NAc, and that this can be inhibited by blockading the glutamatergic synapses in the NAc (Di Ciano and Everitt 2001; McFarland et al. 2003; Park et al. 2002). Kalivas and Volkow (2005) conclude, in an integrative review of the current preclinical and imaging studies that the compulsive character of addicts' drug seeking is caused by long-lasting synaptic changes in the glutamatergic projections from the PFC to the NAc. These synaptic changes result in a decrease in the value of natural rewards and a simultaneous increase in the effect of drug-associated stimuli, and therefore are a key cause of addictive disorders.

3 The Role of Long-Term Potentiation and Neural Plasticity in Addiction

The persistence of addictive behaviour, in particular the long-lasting risk of relapse, presumably depends on the time-stability of implicit drug-associated memories. The question of how memories persist is therefore highly relevant to understanding how addictive disorders work. As with the case of physiological long-term memory (Kandel et al. 2000), we can assume that there are processes on the synaptic level involved. The physical reorganisation of synapses and networks can also be assumed to play a role (Chklovskii et al. 2004). In 1973, Bliss and Lomo found repetitive stimulation of afferent fibres to result in reinforced and longer lasting synaptic transmissions (Bliss and Lomo 1973). The authors called this phenomenon *long-term potentiation* (LTP), and the reverse process *long time depression* (LTD). *Glutamate* and *N*-methyl-D-aspartic acid (NMDA)-receptors play a critical role in the induction of LTP. To date, LTP und LTD are the best-developed model mechanisms explaining the associative modulation of synaptic connections and experience-dependent plasticity within the brain (Malenka 2003; Lüscher and Huber 2010).

Numerous studies have shown an induction of LTP by dopaminergic transmission at the cellular level (Jay 2003). Furthermore, a growing body of evidence suggests that the initiation of sensitization, after exposure to an addictive drug, depends upon the glutamatergic transmission that mediates NMDA-receptor-dependent LTP, which takes place at excitatory synapses in the mesolimbic dopamine system (Kauer 2004). Behaviour sensitization, indicated by increased locomotor reaction on repeated drug-application, is an important animal model that is useful for the study of drug-induced neuronal adaptations relevant for behaviour (Kauer 2004). A 1989 study had already demonstrated that

cocaine-induced behaviour sensitization can be blocked by NMDA-receptor antagonists (Karler et al. 1989). This finding formed the basis for the hypothesis that addictive drugs initiate plastic changes to dopaminergic synapses in the VTA via LTP (Ungless et al. 2001) and that these plastic changes may underlie the persistence of drug-seeking behaviour (Engblom et al. 2008). Several drugs have been demonstrated to cause LTP in the VTA (Saal et al. 2003). Pharmacological blockades of the NMDA receptors in the VTA have been shown to decrease both conditioned place preference induced by cocaine administration as well as behaviour sensitization (Harris and Aston-Jones 2003). Several current studies have demonstrated that addictive drugs induce potentiation of excitatory synapses of dopaminergic neurons in not only the VTA, but also in other brain areas involved in addictive disorders, such as the NAc or PFC (Hyman et al. 2006; Kauer 2004; Kauer and Malenka 2007; Mameli et al. 2009).

4 Novel Treatment Approaches: Cue-Exposure Therapy

Because of the importance of both classical and instrumental conditioning in the development and maintenance of addictive disorders, many have advocated *cue-exposure*-based *extinction training* of conditioned drug-related responses as a potentially effective treatment for addiction (e.g. Drummond et al. 1990; Loeber and Mann 2006). We still know very little about the neurobiological effects of such psychotherapeutic interventions, but this proposed treatment can be compared to existing extinction therapies currently used to treat several other psychiatric disorders. These treatments expose drug addicts to conditioned drug stimuli as a means of reducing drug cravings (Drummond et al. 1990; Monti et al. 1993; Rohsenow et al. 2001; Sitharthan et al. 1997). In these treatments, alcohol-dependent patients might be confronted with their preferred beverages in a controlled environment to help them learn to control their conditioned reactions, and thus to avoid behaviours that could lead to relapse. One meta-analysis evaluated the general effectiveness of this procedure positively (Conklin and Tiffany 2002; meta-analysis by Chambless and Ollendick 2001). The effect sizes for the above-cited studies, regarding a reduced amount of ethanol consumed, including abstinence, vary between $d = 0.17$ (Drummond and Glautier 1994), $d = 0.54$ (Rohsenow et al. 2001); $d = 0.61$ (Sitharthan et al. 1997) and $d = 0.74$ (Monti et al. 1993; for a review, see Conklin and Tiffany 2002). Monti et al. (1993) reported a significant reduction of cravings after cue-exposure treatment, but only for patients who were initially reacting with an urge to drink alcohol when exposed to the sight and smell of an alcoholic beverage. Similar findings were reported by Scheurich et al. (2004). These results suggest that cue-exposure training may only be an effective treatment for patients who already display cue-reactivity before beginning training. In any case, in order to measure the effects of cue-reactivity in humans, one must first define valid read-outs of cue-induced mesolimbic dopaminergic activation. This is especially the case since psychophysiological

reactivity (Drummond and Glautier 1994; Monti et al. 1993; Rohsenow et al. 2001) and self-reported subjective craving (Rohsenow et al. 2001; McCusker and Brown 1995) often do not reliably indicate the effects of exposure (Marissen et al. 2007).

These results taken together suggest that cue-exposure training may represent a viable new therapeutic intervention for addiction.

5 Novel Treatment Approaches: Forced Extinction

Whereas extinction training—including the repeated, unreinforced exposure to stimuli associated previously with drug use—has been in use for some time in the treatment of alcohol dependency (Drummond et al. 1990; Monti et al. 1993; Conklin and Tiffany 2002), pharmacological compounds that strengthen memory consolidation during extinction offer a new treatment option. One of the compounds currently being discussed, D-cycloserine (DCS), an agonist at the glycine binding site of the NMDA receptor, was shown very recently to accelerate reduction of cocaine-induced conditioned place preference in rats (Botreau et al. 2006), suggesting that it might be a useful novel tool to facilitate effects of extinction training on cue-reactivity and cue-induced relapse mechanisms in addiction treatment. To do this, however, requires translating results from animal models into humans, conceptualising pharmacologically facilitated extinction training and adapting the way measures of cue-reactivity are defined.

Whereas the dopamine system mediates cue-induced "wanting," the glutamatergic system seems to be involved in learning processes that mediate sensitization. A growing body of evidence suggests that the beginning of sensitization after exposure to an addictive drug is dependent on glutamatergic transmission mediating NMDA-receptor-dependent LTP at excitatory synapses in the mesolimbic dopamine system (for a review, see Kauer 2004). Such NMDA-receptor-dependent and LTP-mediated synaptic plasticity is known to be one of the basic mechanisms of learning and memory consolidation. NMDA antagonists, when given systemically (Santini et al. 2001) or microinfused into the amygdala (Falls et al. 1992) prevent the extinction of conditioned fear. These findings imply that it might facilitate extinction if it were possible to enhance the functioning of the NMDA receptor. The NMDA-receptor complex is a voltage-dependent, ligand-gated ion channel with a high degree of calcium permeability. Glutamate is an endogenous ligand for this receptor (McBain and Mayer 1994). Glycine also interacts with this receptor as a co-agonist, a function which is necessary for the NMDA receptor to be activated (McBain and Mayer 1994). The compound DCS interacts with the glycine-binding site improving the ability of the NMDA-receptor protein to flux calcium, and initiating a variety of intracellular events critical for learning. The body of preclinical information about these effects of DCS is growing (for a review, see Davis et al. 2006). Currently, two clinical studies have provided evidence that DCS improves the outcomes of exposure therapy. The first study

does so in the context of acrophobia (Ressler et al. 2004), while the second considers social anxiety disorder (Hofmann et al. 2006). One study, conducted on volunteers with higher than normal but still sub-clinical scores on arachnophobia indices (Guastella et al. 2007), failed to detect extinction-facilitating effects of DCS. This study had some notable limitations, however, especially the use of a non-clinical population (students) and the fact that only a single cue-exposure session was administered with DCS. These limitations render the results of this study less comparable to the positive findings in clinical samples.

Whereas earlier studies demonstrated the effects of DCS on extinction processes by using fear conditioning, one recently published study demonstrated that treating rats with 5 mg/kg of DCS was enough to facilitate the extinction of conditioned, alcohol-seeking behaviour, which then also reduced the likelihood of resuming already-extinguished operant responding (von der Goltz et al. 2009). These data suggest that it may be worthwhile to test DCS not only in the treatment of human drug addicts, but also in order to develop new supplementary medications to complement exposure-based psychotherapy by helping extinguish drug-conditioned, appetitive memories.

6 The Theory of Reconsolidation in Addiction

Preclinical addiction studies suggest that the so-called *reconsolidation* theory might provide the basis for a possible therapeutic intervention at the level of protein biosynthesis and subsequent neuroplasticity.

Gene activation and subsequent protein biosynthesis are necessary to consolidate long-term memory, which is then presumed to remain as a stabilized memory trace. Misanin and colleagues challenged this view, proposing that memories become vulnerable to change or loss once they are activated (Misanin et al. 1968). Their work led to the formulation of the reconsolidation hypothesis, which states that reactivation returns already-consolidated memories to a labile state, enabling them to undergo another phase of consolidation, the so-called reconsolidation phase. These reactivated memories are thought to remain unstable for up to six hours (Walker et al. 2003; Duvarci and Nader 2004), behaving like an adaptive update mechanism, thereby allowing new information to be added (Alberini 2005). During this time window, the destabilised memories are thought to be susceptible to disruptions, which could potentially impede their ability to reconsolidate. If this hypothesis is correct, it should open up powerful new avenues for treatment of psychiatric disorders in which maladaptive memories play a major role, such as addiction. A growing body of preclinical studies have used pharmacological techniques to determine the neuronal events that mediate memory reconsolidation, events which include receptors, signal transduction pathways, and proteins. Several preclinical studies have demonstrated memory reconsolidation by using blockers—such as protein-synthesis inhibitors or NMDA-receptor antagonists—to

induce amnesic effects (for review, see Alberini 2005; Tronson and Taylor 2007; Lee 2009).

Using a fear-conditioning paradigm, Nader and colleagues confirmed that after previously conditioned fear memories have been retrieved, these memories can be disrupted with targeted infusions of the protein-synthesis inhibitor *anisomycin* into the lateral and basal nuclei of the amygdala—a site known to play an important role in learning fear (Nader et al. 2000). The study demonstrated that infusing anisomycin shortly after memory reactivation led to amnesia on later tests, while applying anisomycin without first re-exposing the subject to a conditioned cue left memory intact. They therefore concluded that reactivation of a consolidated memory can return it to a labile state from which it must be reconsolidated, as process that, like consolidation, requires new protein synthesis.

While the majority of preclinical research on emotional memory reconsolidation has made use of conditioned aversion paradigms, it was recently demonstrated that retrieved, appetitive, drug-related memories also undergo reconsolidation, which can then be blocked with protein-synthesis inhibitors or NMDA-receptor antagonists. Lee and colleagues, using an animal model with cocaine, were the first to demonstrate this (Lee et al. 2005), and subsequent studies have confirmed their results (Robinson and Franklin 2007; Valjent et al. 2006; Bernardi et al. 2007; Milekic et al. 2006; von der Goltz and Kiefer 2009).

Von der Goltz and Kiefer (2009) later provided evidence that ethanol-associated memories also become unstable and open to being disrupted by protein-synthesis inhibition or NMDA antagonism after being reactivated. Preclinical, behavioural pharmacological experiments such as these have contributed significantly to our understanding of the neurobiological mechanisms involved in memory reconsolidation.

Meanwhile, first support for the reconsolidation hypothesis from human paradigms has been obtained for various memory systems, i.e. motor, declarative and emotional memory. Evidence for the existence of a memory reconsolidation process in rats exposed to *electroconvulsive therapy* was already reported 30 years ago (Misanin et al. 1968), but at the time it remained unclear whether the phenomenon could also be observed under more natural conditions. In 2003, Walker et al. used a finger-tapping paradigm to find that consolidated motor memories, when reactivated, could be returned to a labile state that would again be sensitive to interference. Using interference to block reconsolidation in this way was probably the first convincing demonstration of an erasure of a consolidated memory in humans. Several similar studies followed. Forcato et al. (2007) showed an initially acquired, declarative memory to be impaired when subjects were reminded of the memory material shortly before they learned additional material. Schwabe and Wolf (2010) found evidence that exposing subjects to a stressor directly after memory reactivation could impair how they reconsolidated neutral autobiographical events. The stressor they used was intended to stimulate the release of corticosterone, a glucocorticoid that had previously been shown to block reconsolidation in rodents (e.g. Cai et al. 2006).

Despite these advances, research on potential pharmacological interventions that might help impair memory reconsolidation in humans is still scarce.

Tollenaar et al. (2009) compared the immediate and prolonged effects of a single administration of either *cortisol*, *propanolol* or placebo following the retrieval of previously learned emotional and neutral words. They found retrieval of both emotional and neutral words to be attenuated by cortisol, both immediately as well as after a wash-out period of one week. Propanolol, on the other hand, did not seem to have any effect on declarative memory retrieval in either the short or the long term. Kindt et al. (2009) extended this finding for propanolol by showing it to be effective at erasing conditioned fear-responses in humans, while leaving the declarative memory of the association intact. Despite these encouraging results concerning potential pharmacological interventions in humans, current paradigms still remain to be adapted to separate the well-known effects of cortisol on memory-retrieval from its effects on memory reconsolidation. It is currently still not possible to distinguish impairments in memory retrieval from disruptions in the actual storage process of the maladaptive memories (Nader and Wang 2006; Tronson and Taylor 2007).

At the moment, the majority of studies that have performed experimental disruptions of memory reconsolidation in humans are limited to those using aversive emotional and declarative memory paradigms (Kindt et al. 2009; Soeter and Kindt 2010; Schiller et al. 2010). One particularly relevant question pertaining to possible treatment strategies for addictive disorders is whether appetitive, drug-related memories can be diminished or erased during the process of memory reconsolidation, and if so, which interventions—whether pharmacological and non-pharmacological—might be effective. In a small study with heroin addicts, Zhao et al. (2008) examined the effects of a social stressor presented after retrieval of previously learned, heroin-related words. In the study, increased salivary cortisol indicated that the stressor was effective. The following day, subjects recalled significantly fewer heroin-related words (positive as well as negative), while their memory of neutral words was unaffected. This study gives preliminary evidence that not only can negative emotional memories be impaired by interfering with the reconsolidation process, but so can positive, appetitive memories as well. One difficulty for further research, however, is that most appetitive drug memories underlie implicit conditioning processes, for which study paradigms yet have to be established.

The presented findings have important clinical implications, since they suggest that selectively reducing long-lasting, drug-associated memories could become a viable therapeutic option. Disrupting the reconsolidation of drug-related memories may become an effective treatment strategy for reducing the likelihood of relapse in abstinent addicts.

7 Conclusions

Neural changes induced by chronic substance consumption are important factors underlying the development and persistence of addictive disorders. Implicit learning and memory are both involved in triggering cravings and drug-seeking

behaviour, even after years of abstinence. Results showing the involvement of drug-induced LTP and synaptic changes in the mesolimbic system also support the hypothesis of an addiction memory. Future addiction research will have to integrate the growing amount of knowledge concerning drug-induced neuroplasticity into a valid model of the development of addiction in humans. One of the most important questions facing future researchers is whether addiction memory can be erased using therapeutic interventions. In addition to this need for new pharmacotherapeutic approaches, there is also a need for the development of addiction-specific psychotherapeutic methods which address the implicit contents of addiction memory directly.

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Adolescent Substance Misuse: Neurobiology and Evidence-Based Interventions

Nicola C. Newton, Maeve O'Leary-Barrett and Patricia J. Conrod

Abstract This chapter reviews empirical research on risk-factors for adolescent onset of substance use and misuse, with a particular focus on a recent body of literature aimed at understanding the link between early onset substance use, neuropsychological impairment and future addiction risk. The evidence suggests a causal pathway with some studies showing that adolescents might be more sensitive to the neurotoxic effects of substances, which contributes to their heightened addiction vulnerability. While questions remain pertaining to the extent to which cognitive precursors to early onset substance use account for these impairments, the evidence from a few studies suggests that early substance misuse produces some cognitive or emotional processing impairment beyond these premorbid deficits. The possible interaction between premorbid deficits and the effects of substance use on cognitive development might also explain why early onset substance use so rapidly spirals into substance abuse and dependence and provides a strong rationale for preventing early onset substance use, particularly among those at risk. This chapter then reviews the different approaches to drug and alcohol prevention, the evidence-base for current programs and the essential intervention components that lead to beneficial outcomes and high implementation fidelity.

N. C. Newton (✉)
National Drug and Alcohol Research Centre, University of New South Wales,
22-32 King Street, Randwick, 2052 N.S.W., Australia
e-mail: nickien@unsw.edu.au

M. O'Leary-Barrett
Psychology Department, McGill University, Montreal, Canada

P. J. Conrod
Department of Psychiatry, Université de Montréal, CHU Hôpital Ste Justine, Montreal, Canada

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Contents

1 Introduction 686
2 Aetiology of Substance Use 687
2.1 Risk and Protective Factors for Substance Use 687
3 Adolescent Substance Use and Neuro-Toxicity 688
3.1 Effects of Early Substance Use on Brain Development 690
4 Substance Use Prevention 692
4.1 When and Where Should Prevention Occur? 692
4.2 School is an Ideal Location 692
4.3 Selective Versus Universal Prevention 693
4.4 Effective Principles for School-Based Drug Prevention 695
5 Obstacles to Effective Drug Education in Schools 695
5.1 Internet-Based Interventions 697
6 Conclusions 699
References 699

1 Introduction

Throughout the world, the use of alcohol and other drugs by young people remains high (Australian Institute of Health and Welfare 2008; Babor et al. 2003; Bauman and Phongsavan 1999; Hibell et al. 2007; National Institute on Drug Abuse 2008; Office of National Drug Control Policy 2008). The detrimental effects of substance use are robust and include strains on forming and maintaining healthy relationships, disruption to educational and vocational paths, and an hindrance to overall social development (Chikritzhs and Pascal 2004; Hall et al. 2001; Teesson et al. 2005). In addition, the burden of disease, social costs, and disability associated with substance use is considerable (Begg et al. 2007; Collins and Lapsley 2008; Degenhardt et al. 2008). The peak of this disability occurs in those aged 15–24 years and corresponds with the typical age of initiation of alcohol and drug use (Andrews et al. 2001). Early initiation to substance use is extremely concerning given it is a strong risk factor for the later development of substance use disorders and co-morbid mental health problems (Anthony and Petronis 1995; Behrendt et al. 2009; Grant et al. 2006; Gruber et al. 1996; Teesson et al. 2005). To reduce the occurrence and cost of such problems, preventative interventions need to be initiated early before problems begin to cause disability and harm (Spooner and Hall 2002b). Given that school-based drug prevention is the primary means by which drug education is delivered (Gresham 2004), it is essential to focus on increasing program efficacy. To do this it is first important to understand why drug use is occurring, and then to identify when, where and how prevention should occur.

2 Aetiology of Substance Use

Initiation of drug use by most adolescents is a result of social influences and rebellious behaviors that typically occur during the teenage years. As children move into adolescence they experience increased social, emotional and educational challenges (Simmons and Blyth 2008). This developmental progression coincides with periods of enhanced risk for drug use and access to addictive substances (National Institute on Drug Abuse 2003). It has been suggested that the most promising route to effective prevention of adolescent substance use is to reduce risks factors and enhance protective factors to increase resistance (Hawkins et al. 1992; Spooner and Hall 2002a; Spooner et al. 1996).

2.1 Risk and Protective Factors for Substance Use

Risk factors refer to individual characteristics, variables, or hazards that *increase* the likelihood of an individual developing a disorder, in comparison to the random general population (Arthur et al. 2002). As the exposure to risk factors increases, so does the likelihood of developing substance-misuse problems (Newcomb 1995). Protective factors are factors that *reduce* the likelihood of developing problem behavior, by mediating or moderating the effect of exposure to risk factors (Arthur et al. 2002). There are numerous risk and protective factors that have been implicated in the development of substance use (Brook et al. 2003; Frisher et al. 2007; Hawkins et al. 1992; Loxley et al. 2004; Spooner et al. 1996; Stockwel et al. 2004; Swadi 1999). They can be divided into three main risk factor categories: (1) Genetic factors (predispositions to drug use); (2) Individual factors (characteristics within individuals and their interpersonal environments) and; (3) Environmental/contextual factors (broad societal and cultural factors) (Frisher et al. 2007; Hawkins et al. 1992; Loxley et al. 2004; Spooner et al. 1996; Stockwell et al. 2004).

Genetics factors play an important part in determining vulnerability to drug-seeking and addictive behavior. Evidence including twin studies have shown robust genetic components in alcohol, cannabis, opiate, cocaine, and tobacco addictions, suggesting that a genetic predisposition to substance use problems and addictions are probable (Hawkins et al. 1992; Loxley et al. 2004; Lynskey et al. 2002; Spooner et al. 1996; Volkow and Li 2007).

The individual and interpersonal factors which influence drug use are associated with personality, attitudes, beliefs and early childhood characteristics. Four personality traits associated with early-onset substance misuse are Sensation Seeking, Impulsivity, Anxiety Sensitivity and Hopelessness (Woicik et al. 2009). These traits represent personality-specific motivational pathways to substance misuse (Krank et al. 2011; Woicik et al. 2009), and are also associated with specific drug use profiles (Conrod et al. 2000; Woicik et al. 2009) and patterns of non-addictive psychopathology (Castellanos and Conrod 2006; Mackie et al. 2011).

The internalising traits of Hoplessness and Anxiety Sensitivity have been associated with alcohol consumption for coping purposes. Individuals with high levels of Hopelessness have been found to use substances for self-medication of depression symptoms or the numbing of painful memories (Cooper et al. 1995; Woicik et al. 2009), and are at heightened risk for depressive disorders (Woicik et al. 2009). Anxiety sensitivity refers to a fear of anxiety-related physical sensations due to an unrealistic expectation that they could lead to loss of physical or mental control or other "catastrophic" consequences (Reiss et al. 1986), and is associated with substance use to dampen feelings of anxiety (Comeau et al. 2001). Individuals with high levels of Anxiety Sensitivity are also at increased risk for anxiety disorders (Stewart and Kushner 2001). Impulsivity, on the other hand, is associated with disinhibition over a range of behaviours, including antisocial tendencies (Luengo et al. 1994), problem drinking (Sher and Trull 1994) and polysubstance use (Caspi et al. 1996; Conrod et al. 2010). It is the personality trait most consistently associated with alcohol use disorders (Sher and Trull 1994), and has been associated with early drug experimentation, and severity of drug use (Gerevich et al. 2002). Lastly, Sensation Seeking is related to risk-taking behaviours in general, including heavy alcohol-use for enhancement or social motives (Conrod et al. 2008; Cooper et al. 1995), and is thought to be associated with early onset substance use as a thrill seeking activity. Interestingly, Sensation Seeking is not associated with conduct problems or any other form of psychopathology independent of substance use (Castellanos-Ryan et al. 2011; Conrod et al. 2000; Mackie et al. 2011).

Environmental and contextual factors also play a role in influencing drug use. Particularly, social influence which is recognized as having a strong effect in determining behaviors in adolescents, including drug initiation (Bandura 1977). In particular the perception of drug use as a "normal" behavior, as well as the social acceptability and permissiveness, are good predictors of prevalence of use (Tyas and Pederson 1998). The major environmental factors which influence drug use pertain to peers (Kuntsche and Delgrande Jordon 2006; Oetting and Lynch 2003), family and society (Hawkins et al. 1992; Loxley et al. 2004; Spooner et al. 1996; Stockwell et al. 2004).

3 Adolescent Substance Use and Neuro-Toxicity

Adolescent onset of alcohol and illicit drug use is associated with a myriad of immediate and long-term negative consequences (Zeigler et al. 2005). Onset of alcohol use at or before 14 years of age is strongly associated with increased risk of developing alcohol use disorders, with rates of adult alcohol dependence in this early onset group estimated at 40% (Grant and Dawson 1997, 1998). Adolescent substance use is also associated with greater risk for mental health problems (Merikangas et al. 1998; Rohde et al. 1996), suicidal behaviour (Crumley 1990; Woods et al. 1997), other drug use (Grant and Dawson 1998), poor academic

performance (Wechsler et al. 2000; Zeigler et al. 2005), school drop-out (Wichstrom 1998; Williams and Wynder 1993), risky sexual behaviours (Halpern-Felsher et al. 1996; Tapert et al. 2001a), poor physical health (Clark et al. 2001; Single et al. 2000), and injuries (Hicks et al. 1990). A recent World Health Organization study reported that alcohol use alone accounts for almost 4% of the global burden of health, with deaths attributed to alcohol greater than those caused by HIV/AIDS, violence or tuberculosis (World Health Organization 2011). Moreover, an evaluation of drinking patterns in 73 countries worldwide reported that hazardous and harmful drinking patterns, such as drinking to intoxication and binge drinking, are on the rise among adolescents and young adults (McAllister 2003; The Lancet 2008; World Health Organization, 2008). Compounding this problem are results from major epidemiological studies in the USA (Johnston et al. 2011; Substance Abuse and Mental Health Services Administration 2010) showing that the age of onset of alcohol use has been decreasing over the last 35 years, with youth now initiating alcohol use at 12 years of age on average. Research on other drugs has also shown that the earlier the age of initial use, the greater the chances are of becoming a regular user, developing a dependence, and in turn experiencing the related harms (Behrendt et al. 2009; Patton et al. 2007).

Current theories on how early onset substance use impacts on future risk implicate the effects of alcohol and illicit substances on the adolescent developing brain. There is an extensive literature on the neuropsychological deficits in adolescents and adults with alcohol use disorders, and other substance use disorders. Cognitive impairments have been identified in multiple domains in adult alcoholics and drug users, including verbal and non-verbal performance, learning, memory, abstract reasoning, speed of information processing and efficiency (Beatty et al. 1997; Gottschalk et al. 1982; Miller and Orr 1980). These deficits have been replicated in adolescents with alcohol and substance use disorders, though on a smaller scale. Brown et al. (2000) report a 10% weaker mental performance in alcohol-dependent 15–16 years olds relative to their nondrinking peers. Youth with alcohol use disorders were particularly impaired in tasks involving verbal or nonverbal memory recall (Wechsler 1945). Other studies have reported impairments in verbal and non-verbal memory, attention, executive and visuospatial performance (Tapert et al. 2001b; Tapert and Brown 2000). Sher et al. (1997) found differences in visuospatial ability and motor speed between groups of first-year college students with past year alcohol dependence relative to students with no past-year alcohol use disorder. This study showed that these alcohol-related deficits can be detected in young populations, even when controlling for other confounding factors such as family history of alcohol use disorders. Similarly, spatial working memory deficits are found between alcohol-dependent women and control participants with no history of substance dependence aged 18–25 years (Tapert et al. 2001b).

Cognitive deficits have also been recognised in the non-problematic, social drinking population (Parsons 1998), with the suggestion that there is a continuum of deficits related to quantity of alcohol consumption. One of the most well-controlled investigations is a longitudinal study of neuropsychological functioning

in adolescents assessed prior to initiating drinking and then over a 3-year follow-up, showing that those who transitioned into heavy or moderate drinking showed impaired cognitive function relative to their baseline levels and matched controls who remained nonusers throughout study (Squeglia et al. 2009). Drinking days predicted a 10% reduction in visuospatial task performance from baseline to follow-up in girls and hangover symptoms predicted a 7% reduction in sustained attention for boys. Moderate to high levels of alcohol use and binge drinking may detrimentally affect neurocognitive development, and this study suggests that effects are detectable in the normal, social drinking youth population.

3.1 Effects of Early Substance Use on Brain Development

Adolescence represents a time of maturational change in the brain, and particularly the prefrontal cortex (Chambers et al. 2003; Sowell et al. 1999). The relatively late development of this area is thought to be associated with a salient increase in executive functioning and cognitive control capacity throughout adolescence (Happaney et al. 2004; Spear 2000). A number of studies have noted progressive linear changes between childhood and adulthood in task-specific, (predominantly) prefrontal function during inhibitory and working memory functions (Bunge et al. 2002; Kwon et al. 2002), and reward processing (Casey et al. 2008; Ernst et al. 2006; Galvan et al. 2006), with studies suggesting that adolescence is a unique point in development where inhibitory control is particularly dependent on incentive, particularly reward contingencies (Casey et al. 2008; Ernst et al. 2006; Galvan et al. 2006). Adults and adolescents with histories of substance use show abnormal behavioural and neural activation patterns on tasks of response inhibition and reward sensitivity (Buhler et al. 2010; Castellanos-Ryan et al. 2011; Goldstein et al. 2008; Hester et al. 2005; Reuter et al. 2005). These abnormalities have been shown to be exacerbated during substance withdrawal, and reduced reward-sensitivity has been shown to be restored following presentation of drug cues (Powell et al. 2002), suggesting that these abnormalities result, at least in part, from substance misuse and withdrawal, and might contribute to future addiction vulnerability. Adolescent brains may be particularly susceptible to damage from alcohol use due to the significant neuro-maturation occurring throughout this period (Zeigler et al. 2005). This has been shown to be true in rodent models, where alcohol-neurodegeneration is more severe in adolescent than adult brains (Crews et al. 2000). Ethical considerations in human populations have precluded researchers from experimentally testing the same effect, but results have been mirrored to a certain extent in adolescent populations, where higher rates of nicotine or alcohol dependence are seen despite similar or lower levels of use than adults (Chambers et al. 2003), suggesting heightened adolescent sensitivity.

Because the majority of neuropsychological studies with adolescents are cross-sectional, it has been difficult to conclude whether the observed cognitive abnormalities are causal or consequential to alcohol misuse. There is a large literature

indicating that two major risk factors for adolescent onset alcohol misuse, namely family history of alcoholism and adolescent onset psychopathology (Kirisci et al. 2006), are associated with brain abnormalities that are also seen in adult substance abusers. Functions of inhibitory control, working memory, temporal foresight and delay of reward have been shown to be abnormal in children with disinhibited personalities, childhood disorders of impulsiveness, such as conduct disorder, attention deficit/hyperactivity disorder, and children of alcoholics (Harden and Pihl 1995; Oosterlaan et al. 1998; Peterson et al. 1992; Rubia et al. 2007; Sonuga-Barke et al. 2002). Few studies are able to control for premorbid factors, but those that do show that alcohol-dependent youth with premorbid risk (e.g. familial alcoholism) show particularly impaired neuropsychological function (Tapert and Brown 2000), suggesting an interaction between vulnerability to substance misuse and the effects of substance misuse on the adolescent cognitive development. Current theories of adolescent brain development propose that it is adolescent developmental delay, rather than stable cognitive deficits, that account for the rise in risk taking and attentional difficulties in adolescence and the individual differences seen in these functions (Chambers et al. 2003). Therefore, longitudinal designs that simply apply a pre-post design to control for baseline levels of neuropsychological function prior to onset of substance use might not capture individual differences in how the brain changes over the course of adolescence and how substance misuse and its growth might interfere with such development. Investigations involving multiple testing sessions and growth modeling analyses would be better suited to address this question.

Cannabis remains the most common illicit drug used throughout adolescence (Dubé 2009; Johnston et al. 2011), but there are fewer studies investigating its association with cognitive performance (Pope et al. 2003). Adults with histories of heavy cannabis use show deficits in executive functioning (Fletcher et al. 1996; Solowij et al. 2002), and some studies show that early onset of cannabis use is associated with lower cognitive abilities later in life (Ehrenreich et al. 1999; Wilson 1998). As alcohol and other drug use often go hand in hand (Grant and Dawson 1998), we may therefore expect similar associations with neurocognitive functioning in adolescence.

The evidence reviewed suggests that early onset substance abuse is associated with neuropsychological impairment and future addiction risk. There is also some suggestion that adolescents might be more sensitive to the neuro-toxic effects of substances, which contributes to their addiction vulnerability. While questions remain pertaining to the extent to which cognitive precursors to early onset substance use account for these impairments, evidence from a few studies suggests that adolescent onset substance misuse produces some cognitive or emotional processing impairment beyond these premorbid deficits. The possible interaction between premorbid cognitive deficits and the effects of substances on cognitive development might also explain why early onset use so rapidly spirals into substance abuse and dependence as well as a myriad of other mental and physical problems. Preventing early onset substance use could therefore potentially have a

broader effect on adolescent outcomes, including protecting adolescent cognitive development as well as the development of future addictions.

4 Substance Use Prevention

4.1 When and Where Should Prevention Occur?

Adolescence and young adulthood coincide with the occurrence of critical developmental periods in terms of social and emotional wellbeing (Simmons and Blyth 2008; Spooner et al. 1996). It is a time when young people move toward independence and autonomy, decrease dependence on families and schools, and place more emphasis on acceptance by peers. For most young people, this progression to adulthood is positive. However, this transition is also the time when risk-taking behaviour is high and vulnerability to mental illness and substance-use disorders is at its peak, which, if left untreated, can be lifelong and cause severe disability (Andrews et al. 2001). As outlined above, coinciding with these social and emotional influences is the ongoing development of the brain which continues well beyond childhood and adolescence (Sowell et al. 2004; Tapert et al. 2005). The late development of the prefrontal cortex may reduce an adolescents' ability to carry out intended and planned choices (Luna and Sweeney 2004), and can exaggerate the brain's responses to immediate rewards (Galvan et al. 2006). The deleterious effects of alcohol and illicit drugs may be particularly noticeable in adolescents who begin to use substances early, due to potential neurotoxic effects on brain functioning, in particular the developing prefrontal system.

In light of the above findings, it seems important that prevention programs be introduced in the early adolescent years. Ideally, prevention should be implemented prior to initial exposure to drugs and before the social and emotional influences come into full effect to reduce the adverse impacts from drug use on the developing brain and reduce potential harms. Implementing programs early will ensure young people are provided with the knowledge and skills they need to make responsible and informed decisions regarding their drug use (Dielman 1995). Schools offer the ideal location to do this.

4.2 School is an Ideal Location

School-based drug education offers numerous advantages over other prevention approaches such as family- or community-based interventions. Attending school is a mandatory requirement in most Western countries and it is at school where young people spend over a quarter of their waking lives (Cuijpers 2002). Hence, schools offer a location where educators are able to reach large audiences at one

time whilst keeping costs low (Botvin 1999, 2000; Cuijpers 2003; Gottfredson et al. 1996; Jones et al. 2006; Shin 2001; Wenter et al. 2002).

Not only is school a place where peer interaction (a significant risk factor for drug use) is high, it also coincides with a time when young people are beginning to experiment or are exposed to drugs (Australian Institute of Health and Welfare 2008; Botvin and Griffin 2003; Sharma 2006). Therefore, schools provide a context to deliver preventive interventions before harmful use begins (Berkowitz and Begun 2003). Evidence suggests that drug education is best taught in the context of sequential and developmentally appropriate stages, and the school health curriculum provides the ideal context to do this (Ballard et al. 1994; Dusenbury and Falco 1995; Meyer and Cahill 2004). In addition, students have rated school-based programs as significantly more effective than other forms of prevention, such as television advertisements and billboards, in preventing them from using drugs and encouraging them to seek help if they do have a problem (Lisnov et al. 1998). Overall, school-based drug education is appealing to both students and educators because it offers both practical and economic advantages and can be tailored to different development stages (McBride 2003).

4.3 Selective Versus Universal Prevention

There are two common approaches to school-based drug education: the ‘selective approach’ and the ‘universal approach’ (Offord 2000). The selective approach involves developing and delivering prevention programs to target specific populations, such as individuals at greatest risk for developing substance use problems. Selective interventions have the advantage of allowing the focus of limited resources to be used on those most at need. They also address individual needs of homogeneous at risk groups and offer an opportunity to tailor interventions to the etiological processes implicated in different risk profiles (Conrod et al. 2006, 2008, 2010). Selective prevention programs are often overlooked due to their practical limitations. It is not only difficult to initially identify those individuals at greatest risk, but finding suitable, cost-effective ways to screen and deliver interventions can also be challenging (Offord 2000). However, in recent years we have seen the development of selective programs which are showing that these ethical and practical obstacles can be overcome.

One such approach, known as Preventure, is a brief, selected program that presents a novel approach to substance misuse prevention by targeting personality risk-factors for early-onset drinking or illicit drug use. It is the first and only school-based alcohol and drug prevention program that has been shown to prevent growth in alcohol and substance-misuse in three separate trials across Canada (Conrod et al. 2006) and the United Kingdom (Conrod et al. 2008, 2010, 2011; O’Leary-Barrett et al. 2010), through targeting youth with elevated scores on four personality risk-factors for early-onset substance-misuse and other risky behaviours: Hopelessness, Anxiety-Sensitivity, Impulsivity and Sensation-Seeking

(Krank et al. 2011; Woicik et al. 2009). Youth are screened in classroom settings during school hours, and those scoring one standard deviation above the school mean on one of these four personality traits, as measured using the Substance Use Risk Profile Scale (Krank et al. 2011; Woicik et al. 2009), are invited to participate in coping skills workshops. Each of the four personality-specific interventions involve adolescents selected for specific personality profiles to work together over two 90-minute group sessions guided by a trained facilitator and co-facilitator at school. The interventions are manualised and incorporate psycho-educational, motivational enhancement therapy and cognitive-behavioural components, and include real life 'scenarios' shared by high-risk youth in specifically-organised focus groups. A novel component to this intervention approach is that all exercises discuss thoughts, emotions and behaviours in a personality-specific way.

Three separate randomised-controlled trials have shown that this intervention approach is associated with reduced drinking, binge drinking and problem drinking symptoms in high-risk youth over 6-months (Conrod et al. 2006, 2008, 2010; O'Leary-Barrett et al. 2010), with one of these trials showing 2-year reductions in problem drinking symptoms and illicit drug use in high risk youth (Conrod et al. 2010). A recent cluster-randomised trial known as Adventure has replicated the preventative effects of personality-targeted interventions on alcohol use when delivered by trained school-staff (Conrod et al. 2011; O'Leary-Barrett et al. 2010), thus suggesting that this intervention approach can operate within an implementation model that has a higher likelihood of being adopted by schools in a sustainable manner. The results of the Adventure program are central to the development of an effective (as opposed to merely efficacious) intervention. This trial demonstrates that targeted interventions can be successfully delivered by educational staff who have been trained and supervised, and has the potential to become a sustainable school-based prevention model.

Effect sizes for binge-drinking from the Adventure trial were similar to those from previous clinician-run personality-targeted intervention trials, with Odds Ratios (OR) between 0.4 and 0.5 across all trials for youth who had already consumed alcohol by 13 years of age (i.e. a particularly high-risk group). These ORs correspond to a 50–60% decreased likelihood of having binge drank 6 months post-intervention. The corresponding ORs for a sample including youth who were non-drinkers at baseline were 0.65–0.7, representing a 30–35% decreased likelihood of reporting binge drinking 6 months. Numbers Needed To Treat (NNTs) across the 3 trials for baseline alcohol users ranged from 4 to 6, indicating that 4–6 individuals are required to receive an intervention in order to prevent one case of binge drinking. These effect sizes are remarkable given that the most effective universal alcohol prevention programmes have NNT values from 9 to 30 (Foxcroft et al. 2002), which require targeting double the number of adolescents in order to prevent one case of binge-drinking.

Universal prevention on the other hand, addresses the entire population within a particular setting (e.g. school), regardless of their level of risk for drug use (Mrazek and Haggerty 1994). The aim of universal interventions is to delay the onset of substance use by equipping individuals with the information and skills

they need to prevent use. In schools, universal programs focus largely on teaching awareness education (knowledge and harms), normative education, social and drug resistance skills and promoting positive peer relationships. Universal programs offer the advantage of being delivered on large scales and as such, they have the potential ability to reduce substance use and harm to a greater audience (Jones et al. 2006; Midford 2008). Importantly they avoid the risk of stigmatising individuals, which is imperative, given the sensitive nature of drug use and risk (Offord 2000). Although effect sizes of universal programs are generally more modest than selective or indicated programs, they can still provide important and significant cost-benefits.

A recent review of school-based universal prevention has identified a number of effective programs, all of which incorporate a social influence or developmental approach to prevention (Foxcroft and Tsertsvadze 2011). These include the Life Skills Training program (Botvin et al. 2001, 2003), the Unplugged program (Faggiano et al. 2008, 2010), the Climate Schools program (Newton et al. 2009, 2010; Vogl et al. 2009) and the Good Behaviour game (van Lier et al. 2009). Regardless of the approach, the effective components of school-based prevention programs are the same.

4.4 Effective Principles for School-Based Drug Prevention

The development and evaluation of school-based prevention programs intended to prevent substance use has significantly increased over the past few decades. The number of systematic reviews and meta-analyses examining the effectiveness of school-based drug prevention continues to grow. These reviews have consistently established that school-based prevention can result in significant increases in knowledge about substances and improved attitudes towards substance use (Botvin 2000; Botvin and Griffin 2007; Faggiano et al. 2008; Hansen 1992; Midford et al. 2001; Roona et al. 2000; Soole et al. 2005; Tobler et al. 1999, 2000). However, they have not been able to consistently demonstrate the effectiveness of school-based drug prevention in reducing actual substance use (Botvin and Griffin 2007; White and Pitts 1998). Table 1 summaries the principles that have consistently been associated with effective drug prevention programs in schools (Ballard et al. 1994; Cuijpers 2002; Dusenbury and Falco 1995; Meyer and Cahill 2004; Midford et al. 2002).

5 Obstacles to Effective Drug Education in Schools

Although effective school-based prevention programs do exist, there are also many barriers or ‘obstacles’ which can impede program effectiveness (Botvin 2004; Dusenbury and Hansen 2004; Elliott and Mihalic 2004; Kaftarian et al. 2004).

Table 1 Effective principles of school-based prevention for substance use

• Evidence-based and theory driven
• Acknowledge and target risk factors for substance use and psychopathology
• Present developmentally appropriate information
• Implemented prior to harmful patterns of use are established
• Be part of a comprehensive health education curriculum
• Adopt a social influence or comprehensive approach to prevention and:
–Provide resistance skills training, and
–Incorporate normative education
• Content is of immediate relevance to students
• Use peer leadership, but keep teacher as the central role
• Address values, attitudes and behaviours of the individual and community
• Sensitive to cultural characteristics of target audience
• Provide adequate initial coverage and continued follow-up in booster sessions
• Employ interactive teaching approaches
• Can be delivered within an overall framework of harm minimization

Arguably the greatest obstacles to effective school-based drug prevention can be attributed to issues regarding implementation and dissemination of programs (Cahill 2007; Castro et al. 2004; Dusenbury and Hansen 2004; Ennett et al. 2003; Greenberg 2004; Pentz 2004; Rohrbach et al. 1996).

The dissemination of drug prevention programs into schools is not always entirely successful (Botvin and Griffin 2003, 2007; Cuijpers 2003). Specifically, Ennett and colleagues (2003) found that only 14% of schools in the US implemented evidence-based programs, i.e. programs which incorporate correct content and delivery as identified in the literature as having the largest effect sizes in reducing drug use (Tobler et al. 2000). It is possible that because evidence-based programs are rarely designed and packaged in ways that are competitive with commercial programs and, once funded trials of prevention cease, schools do not have sufficient motivation or resources to continue using such programs (Cuijpers 2002; Cuijpers et al. 2002; McBride et al. 2000; Wenter et al. 2002). It could also be a result of the many challenges that arise when implementing prevention programs into the classroom. This is known as 'implementation fidelity' (Botvin 2004; Botvin and Griffin 2003).

Implementation fidelity refers to adhering to, and implementing, a program in the exact way it was designed to be (Dane and Schneider 1998). A large study examining the implementation fidelity of substance use prevention programs indicated that one-fifth of teachers reported not using a curriculum/program guide at all, and only 15% reported following one very closely (Ringwalt et al. 2003). This is of great concern because research shows implementation fidelity is linked with the effectiveness of programs. Specifically, programs delivered with high fidelity lead to superior outcomes for students, and programs delivered with poor fidelity lead to poorer outcomes for students (Dane and Schneider 1998; Elliott and Mihalic 2004). Internet-based technology offers a practical means of delivering evidence-based programs whilst assuring implementation fidelity.

5.1 Internet-Based Interventions

Internet-based technology offers many advantages over traditional methods of delivering prevention programs. Programs delivered over the Internet require minimal teacher training and input, guarantee complete and consistent delivery of the content of a program, and are both feasible and scalable to meet the needs of large audiences. In addition, the Internet offers a way of updating information with ease; therefore, after the initial development costs, internet-based resources offer a cost-effective means for delivering and disseminating prevention. In comparison to traditional teaching methods, the use of computer technology in education has been shown to accelerate learning and improve educational achievement and outcomes (Barber 1990; Bosworth 2003). Computers also allow students to learn material at varied paces, provide them with immediate feedback, and allow students to learn information and skills with relative anonymity, which is important given the sensitive nature of drug use (Bosworth et al. 1994).

In recent years, promising research has been conducted into the development and evaluation of interventions delivered by computers or over the Internet to reduce substance use in adolescents. Computer-based drug prevention programs for adolescents generally involve young people navigating their way through simulated real-life situations involving characters and contexts to which they can relate (Gregor et al. 2003; Schinke et al. 2004). The current range of youth drug prevention programs are both brief (Duncan et al. 2000; Gregor et al. 2003) and intensive (Gropper 2002; Schinke et al. 2004, 2005; Williams et al. 2005) and have been designed for both universal (Duncan et al. 2000; Gregor et al. 2003; Gropper 2002; Schinke et al. 2004; Williams et al. 2005) and targeted populations (Bosworth et al. 1994; Schinke et al. 2005). From the evidence that exists, it appears that such programs are both feasible and acceptable (Bosworth et al. 1994; Duncan et al. 2000; Gregor et al. 2003; Schinke et al. 2004, 2005; Williams et al. 2005).

In terms of efficacy, computerised drug prevention programs for youth have been shown to increase drug-related knowledge (Gropper 2002; Marsch et al. 2007; Newton et al. 2009a, 2009b, 2010; Vogl et al. 2009), decrease pro-drug attitudes (Gropper 2002; Schinke et al. 2004; Vogl et al. 2009; Williams et al. 2005), increase drug resistance skills (Duncan et al. 2000), increase anxiety management skills (Williams et al. 2005) and decrease reported intention to use drugs (Duncan et al. 2000; Gregor et al. 2003). The evidence for behavioural change is more limited as most studies have failed to collect behavioural measures (Duncan et al. 2000; Gregor et al. 2003; Gropper 2002). From those that have collected measures of behavioural change, the results are promising.

One Internet-based program which has demonstrated positive effects in reducing actual drug use is the series of Climate Schools programs for drug prevention specifically designed to overcome factors which compromise program efficacy. The modules are contemporary cartoon-based educational programs based on a social influence approach to prevention and are consistent with the effective harm minimisation framework (McBride et al. 2006). The programs are

designed to fit within the school health curriculum and are facilitated by the Internet thereby guaranteeing complete and consistent delivery whilst ensuring high implementation fidelity (Schinke et al. 2004). This interactive classroom-based approach to prevention is therefore feasible, scalable and easy to implement.

Each Climate Schools module consists of 6 × 40 min lessons. The first half of each lesson is completely individually online where students follow a cartoon storyline of teenagers experiencing real life situations and problems with alcohol and cannabis. The cartoon storylines are used to engage and maintain student interest and involvement over time (Schinke et al. 2004). The second part of each lesson is a predetermined activity delivered by the teacher to reinforce the information learnt in the cartoons.

The efficacy of the Climate Schools model has been demonstrated for stress reduction (Van Vliet and Andrews 2009) and alcohol misuse (Newton et al. 2009; Vogl et al. 2009). In one or both studies the Climate Schools: Alcohol module was more effective than usual classes in increasing alcohol related knowledge, decreasing positive expectancies about alcohol, decreasing average alcohol consumption, frequency of binge drinking (drinking in excess), and alcohol related harms.

The most recent Climate Schools program to be developed and evaluated was the Climate Schools: Alcohol and Cannabis course. This course comprises the delivery of the Climate Schools: Alcohol Module followed six months later by the delivery of the Climate Schools: Alcohol and Cannabis Module to reinforce the material taught in the Alcohol module and transfer the knowledge and skills to the use of the most commonly used illicit drug, cannabis. This aim of the Climate Schools: Alcohol and Cannabis course is to decrease alcohol and cannabis use, and related harms in 13–14 year olds. To evaluate the effectiveness of the course, a cluster randomised controlled trial was conducted with 764, 13-year olds from ten Australian secondary schools in 2007–2008. Half the schools were randomly allocated to the computerised prevention program (n = 397), and half to their usual health classes (n = 367). Participants were assessed at baseline, immediately post, and at six and twelve months following the intervention. Compared to the control group, students in the intervention group showed significant improvements in alcohol and cannabis knowledge at end of the course and the six- and twelve-month follow-ups. In addition, the intervention group showed a significant reduction in average weekly alcohol consumption and frequency of cannabis use at the six month follow-up and a reduction in average weekly alcohol consumption, and frequency of drinking to excess at the 12-month follow-up.

The findings from the robust evaluations of the Climate Schools drug prevention programs provide evidence that this innovative new platform can not only increase drug related knowledge and decrease positive attitudes towards drugs but it can also reduce actual use of alcohol and cannabis (Newton et al. 2009a, 2009b, 2010; Vogl et al. 2009). Such positive effects, together with the numerous implementation advantages and high fidelity associated with computerised delivery, suggest the Internet now offers a promising delivery method for preventing substance use in adolescents.

6 Conclusions

The evidence reviewed highlights the neurotoxic impact of early onset substance use on the adolescent brain, and the concurrent and prospective impact on neurocognitive functioning, whilst highlighting the need for prospective studies to disentangle the causal relationships between risk for and impact of early substance use. This chapter has also emphasised the heightened risk of future abuse and/or dependence resulting from early onset use, and both the concurrent and prospective risks of mental health problems, and social and vocational disadvantages related to sustained substance use. These studies therefore underlie the importance of early prevention and intervention programs, and the necessity of an evidence-based approach. Given that school-based prevention is the primary means by which alcohol and other drug education is delivered, it is essential to focus on increasing program efficacy. Ideally, preventive interventions should be based on either a social influence or comprehensive approach to prevention, should use interactive delivery techniques, be age and context appropriate, be taught in the context of sequential stages, and make use of peer leaders. Over the past decade, the array of school-based prevention programs for alcohol and other drug use has significantly increased and programs are starting to demonstrate effects in reducing actual substance use. Despite the existence of such programs, many educators continue to implement programs that have not been evaluated or which fail to show behavior change. If the aim is to reduce substance use and the associated detrimental harms, it is imperative that schools and educators adopt only those programs which are evidence-based and that future developments are driven from what we know works.

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Deep Brain Stimulation as a Therapy for Alcohol Addiction

Thomas F. Münte, Hans-Jochen Heinze and Veerle Visser-Vandewalle

Abstract Deep brain stimulation (DBS) has been firmly established as a therapy for movement disorders. Recently, evidence from case reports and small case series also suggests DBS to be effective in psychiatric disorders including addiction. Here we review the rationale of DBS in addiction and the selection of possible targets. We then consider evidence from animal models as well as human case studies. We conclude that DBS in particular of the nucleus accumbens (NAcc) represents a promising treatment option in addiction which deserves further investigation.

Keywords Deep brain stimulation · Nucleus accumbens · Alcohol addiction · Incentive salience · Reward processing

Abbreviations

DBS	Deep brain stimulation
LFP	Local field potentials
ERP	Event-related potentials
NAcc	Nucleus accumbens
SNc	Substantia nigra pars compacta
VTA	Ventral tegmental area

T. F. Münte (✉)
Clinic for Neurology, Ratzeburger Allee 160, 23538 Lübeck, Germany
e-mail: Thomas.muente@med.ovgu.de

H.-J. Heinze
Department of Neurology, University of Magdeburg, Magdeburg, Germany

V. Visser-Vandewalle
School for Mental Health and Neuroscience, Maastricht Institute for Neuromodulative Development, Maastricht University and Medical Centre, Maastricht, The Netherlands

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Contents

1 Introduction 710
2 Neurosurgical Interventions in Alcohol and Drug Abuse 710
3 Deep Brain Stimulation 712
3.1 Principles 712
3.2 Established Uses 712
3.3 Possible Mode of Action of DBS in Addiction 712
3.4 Animal Models 714
3.5 Risks 716
4 Available Evidence on DBS in Alcohol Dependence 716
5 Deep Brain Stimulation as a Window into the Addicted Brain 718
6 Ethical Issues 722
7 Perspectives 723
References 723

1 Introduction

Deep brain stimulation (DBS) had initially been introduced for the treatment of movement disorders such as Parkinson's disease and dystonia more than 20 years ago. Briefly electrodes are implanted to stereotactically targeted areas of the brain and connected to a stimulation device positioned under the pectoral muscle. Trains of pulses are delivered via these electrodes that are thought to interfere with the function of the target area. Besides its undisputed place in the treatment of advanced stage movement disorders, DBS has recently been used to treat a variety of psychiatric disorders such as obsessive–compulsive disorder (OCD) (Anderson and Ahmed 2003; Denys et al. 2010; Greenberg et al. 2006; Sturm et al. 2003), Tourette syndrome (Mink 2009; Porta et al. 2009; Servello et al. 2008) and depressive disorder (Bewernick et al. 2010; Lozano et al. 2008; Malone et al. 2009; Mayberg et al. 2005; Schlaepfer et al. 2008). More recently, addiction has also been proposed as a potential area in which DBS might be beneficial. In this review, we will first consider earlier neurosurgical approaches to the treatment of addiction and will then give an overview about the status of DBS in addiction.

2 Neurosurgical Interventions in Alcohol and Drug Abuse

Prior to the advent of DBS, the repertoire of functional neurosurgery was mostly restricted to applying strategic lesions to alleviate neuropsychiatric conditions. For example, cingulotomy, i.e., a lesion of the cingulate gyrus, was used to treat depression, anxiety, obsessive–compulsive disease, and drug addiction (Ballantine et al. 1987; Christmas et al. 2004; Laitinen 2001; Mashour et al. 2005). The results of

this procedure with regard to drug addiction and abuse have been mostly favorable: In a study of 28 patients addicted to opiods (Balasubramaniam et al. 1973) treated with bilateral stereotactic cingulotomy, 22 patients were reported as successfully treated, staying abstinent during the follow-up period which ranged from 4 to 24 months. Reported side effects included extreme self-confidence and affective changes. In another study, opiate craving was abolished after bilateral anterior cingulotomy in three patients with chronic and otherwise intractable pain. One of the three showed a transient flattening of affect. Another large series of 73 patients addicted to either opiates or alcohol and treated by stereotactic lesions of the anterior cingulate gyrus was reported by Kanaka and Balasubramaniam (1978). This study used a rather strict criterion for treatment success, as patients ingesting alcohol or opiates at least once after surgery were considered failures. Using these criteria, 68% of the alcohol addicted patients and even more of the opiate addicts had a successful treatment. A paper on an even larger series of 348 patients with heroin addiction of 2–15 years duration reported a reduction or even absence of drug craving at the time of discharge (Medvedev et al. 2003). Longer term success-rate was 62%, comprising 45% of patients who completely abstained from drugs and 17%, who had one relapse. The papers discussed so far mainly concentrated on gross markers of clinical success. They are less outspoken with regard to side effects of cingulotomy. A recent paper on the effects of bilateral cingulotomy on neurocognitive function revealed, however, that language, memory, motor, visual-constructional, and intellectual functions were unaffected. An impairment of focused attention was seen during an early post-operative assessment (Yen et al. 2009). These patients were treated because of intractable pain and not for drug addiction, however. Case reports of patients receiving cingulotomy because of drug problems pointed to executive impairments (Cohen et al. 1999; Lenhard et al. 2005).

Another target that has been tried in alcohol and drug addiction has been the hypothalamus, in particular the ventromedial part. Following an early case report (Muller et al. 1973) and studies in animals (Kerr and Pozuelo 1971), this procedure was performed uni- or bilaterally in 15 patients with alcohol and drug addiction. While there were some effects on drug intake and self-control in general, these were outweighed by severe neurocognitive problems including diminished drive, amnesia, and vegetative symptoms.

Another target for stereotactic neurosurgery has been the Substantia Innominata (Knight 1969). A small case series of five heroin addicts found that withdrawal symptoms were alleviated thus allowing patients to abstain from the drugs. There are no further data on the substantia innominata with regard to stereotactic neurosurgery and addiction.

A recent report from China (Gao et al. 2003) focused on the ablation of the NAcc in 28 patients who had been drug addicts for at least 3 years. The rationale for this target came from animal and human experiments which had revealed a relationship between craving and the mesocorticolimbic dopamine system. Blocking the response of the NAcc to drugs and drug-conditioned cues should prevent craving for drugs and in this way cause reduction in the relapse rate. Bilateral lesions were placed in the core of the NAcc. Mean follow-up was 15 months. Complete remission was

reported in seven patients. Ten further patients relapsed within 6 months, but showed less severe withdrawal symptoms. Of the remaining 11 patients, two had poor outcome, seven were not included in the analysis for various reasons and two were lost for follow-up. Side effects included temporary personality change ($n = 2$) and temporary memory problems ($n = 4$). These authors concluded that bilateral ablation of the NAcc is both safe and effective in drug addiction.

3 Deep Brain Stimulation

3.1 Principles

DBS is achieved by the implantation of a stimulation system comprising the electrode lead that is placed into the target structure using a stereotactic frame and a pulse generator implanted under the pectoral muscle similar to a cardiac stimulating device (Deuschl et al. 2006). The electrode lead usually is insulated in polyurethane and has a tip with four platinum iridium electrodes. Recently, multi-electrode leads have been introduced. Electrode lead and stimulating device are connected by an insulated wire that runs from the head, down the side of the neck to the site of the stimulator. High frequency stimulation with frequencies of around 130 Hz, a pulse width of about 60 μs, and a stimulation strength of around 1.5 mA is most often used. Bipolar as well as unipolar stimulation protocols are used. The electrode contact as well as all other parameters used for stimulation can be changed by the clinician using an external control device. Obviously, parameter setting is much easier in movement disorders with clear and objective target symptoms such as tremor or akinesia.

3.2 Established Uses

DBS devices have been approved by the Food and Drug Administration (FDA) of the USA for the treatment of essential tremor and Parkinson's disease. It is also approved under a humanitarian device exemption for dystonia. Numerous other applications have been described over the past 10–15 years (see Table 1). However, data on most of these applications still are sketchy and far from conclusive. This also pertains to the possible use of DBS in addiction. Up to now only a few animal studies and several case reports and small case series in humans have been published.

3.3 Possible Mode of Action of DBS in Addiction

The precise effects of high frequency DBS on the cellular and circuit levels are not known and several contradicting hypotheses have been proposed in the literature.

Table 1 Target structures for DBS in different neuropsychiatric conditions

Structure	Disorder
Ncl. subthalamicus	Parkinson's disease (PD)
Globus pallidus int.	Dystonia, PD
Ventral intermediate part of the thalamus	Tremor, PD
Centre median nucleus/ parafascicular (CM/PF complex)	PD
Unspecific thalamocortical system	Minimally conscious state
Pedunculopontine nucleus	PD
Zona incerta	PD
Posterior inferior hypothalamus	Cluster headache, obesity
Ncl accumbens	OCD, depression, addiction

Beurrier et al. (2001) suggested that depolarization blockade might interrupt activity of the subthalamic nucleus (and by extension other target structures). This proposal was based on experimental findings that a 1-min tetanic stimulation at 100–250 Hz led to a full blockade of ongoing subthalamic nucleus activity which outlasted stimulation for about 6 min. This effect was thought to be mediated by an influence on voltage-gated currents. An account based on synaptic inhibition was proposed by Dostrovsky and Lozano (2002). These authors suggested that stimulation leads to a release of GABA and consequently to the inhibition of downstream neurons. Feuerstein et al. (2011) have analyzed the physiological commonalities of target sites stimulated in different neuropsychiatric conditions and have suggested that DBS effects and side effects may sufficiently be explained by selective GABA release. Urbano et al. (2002) suggested that high frequency stimulation might lead to transmitter depletion thereby preventing efferent output of stimulated neurons, an account that has been dubbed synaptic depletion hypothesis. Other authors have tried to explain the effects of DBS at the network rather than the cellular level (Montgomery and Baker 2000). For example, Hammond et al. (2008) suggested that DBS corrects the spontaneous pathological patterns of neuronal networks. They reasoned that extracellular stimulation activates subsets of both afferent and efferent axons, leading to antidromic spikes that collide with ongoing spontaneous ones as well as orthodromic spikes that evoke synaptic responses in target neurons. Therefore DBS might interfere with spontaneous pathological patterns by introducing a regular activity in several nodal points of the network.

The rationale of performing DBS within the NAcc is based on the role of this brain site in processing of rewards and instantiation of goal-directed actions. Reward-related behavior implies selection among different alternatives: Affective experiences result in positive or negative evaluations of the events associated with these experiences. Sensory representations of these events thereby become salient and attention-attracting and thus are preferentially processed. This results in actions that increase (or decrease) the probability of such events to reoccur. Importantly, the contingency of action and reward can be extended to action sequences, allowing the execution of several successive actions with only the last in the sequence being rewarded. Such sequences have been modeled as Actor-Critic interactions with

temporal-difference (TD) learning algorithms. According to this view, each action results in a difference between its actual and its expected value. This difference is called the "prediction error" and is used to correct the value associated with the previous state (Schultz et al. 1997). The 'Critic' assesses the prediction error to yield an optimal action strategy, i.e. minimizing punishments and maximizing rewards, which is implemented by the 'Actor' (Takahashi et al. 2009).

Importantly, the Actor-Critic model can explain fundamental aspects of addictive behavior. Moreover, the components of this model (Actor, Critic, prediction error) can be assigned to processes and structures in the basal ganglia and the midbrain (Takahashi et al. 2009). Phasic changes of the activity of dopaminergic neurons in the substantia nigra pars compacta (SNc) and in the ventral tegmental area (VTA) show properties expected for a prediction error signal: an unexpected reward (i.e. a positive prediction error) leads to an increase, the omission of an expected reward (negative prediction error) to a decrease in the firing of such neurons. Moreover, an expected reward does not elicit an activity change of dopaminergic neurons, if preceded by a cue stimulus. In this situation, dopaminergic activity can be observed in response to the cue-stimulus (Schultz et al. 1997; Knutson et al. 2001).

The mesolimbic as well as the nigrostriatal pathway fulfill the functions of Critic and Actor, respectively. Hence, the NAcc receives input from the basolateral amygdala, the hippocampus, and medial prefrontal areas and projects to the VTA/SNc which in turn projects back to the NAcc as well as to the dorsal striatum. This allows the NAcc to integrate information about past and current affective states as well as action-dependent changes and to send this information to the dorsolateral striatum which also receives dopaminergic input from the SNc as well as from the dorsolateral prefrontal and lateral orbitofrontal cortex. The dorsal striatum projects to the pallidum and to the thalamus and can thus influence action selection (Siessmeier et al. 2006).

The Actor/Critic model suggests that an impaired evaluation by the Critic might be at the core of addictive behavior. In this case, the prediction error might reflect expectation and success with regard to the intake of the addictive substance. This assumption has been substantiated by clinical data: alcohol dependence changes evaluation processes in humans (Wrase et al. 2007) and leads to structural and functional changes of the NAcc, i.e., to volume reduction and to a reduction of the availability and sensitivity of D2 receptors (Heinz et al. 2004, 2005).

Dysfunctions of the NAcc have also been proposed to underlie deficient cognitive control in cue-induced craving (Heinz et al. 2009). In the light of these considerations, interfering with the function of the NAcc by DBS might ameliorate cue-induced craving and thus prevent relapse in alcohol addiction.

3.4 Animal Models

A small number of studies has been conducted to examine the effects of DBS in animal models of addiction. With specific reference to the possible clinical application of DBS of the NAcc in alcohol dependence, Henderson et al. (2010)

examined whether this procedure leads to decreases in alcohol intake in alcohol-preferring (P) rats. P rats are selectively bred to spontaneously consume alcohol in rather large quantities (Bell et al. 2006). A specific feature of these animals is that they increase alcohol intake after a period of deprivation, an effect that is also observed in human alcoholics. P rats with a stable alcohol intake of about 5–7 g/day over approximately 2 weeks were treated with DBS (140–150 Hz, 60 μs pulse width, and 200 μA current intensity) and alcohol intake and alcohol preference were assessed. DBS of the NAcc reduced alcohol consumption in P rats both acutely and after a period of alcohol deprivation. While the experiment of Henderson et al. (2010) did not allow a mechanistic interpretation of the observed findings, the authors nevertheless concluded that DBS in the NAcc is efficient in reducing alcohol intake in an animal model of alcohol abuse and that similar effects might be expected in patients with alcohol addiction.

Knapp et al. (2009) studied the differential effects of stimulation of the core or shell region of the NAcc on alcohol intake in rats specifically conditioned to drink. Alcohol consumption was significantly reduced from baseline levels regardless of whether shell or core was stimulated. Water consumption remained unchanged pointing to a specific effect of DBS on ethanol intake in the rat. However, it is important to note that in both preclinical DBS alcohol studies an acute stimulation paradigm was used which makes the translation of these data difficult as in the human condition chronic stimulators are used.

Vassoler et al. (2008) used an animal model of cocaine relapse (cocaine priming-induced reinstatement of drug seeking) to examine the influence of bilateral DBS of the shell of the NAcc. Cocaine at a dose of 10 or 20 mg/kg led to reinstatement of cocaine seeking which was significantly attenuated by DBS. In control experiments it was shown that DBS of the dorsal striatum did not stop reinstatement of cocaine seeking. Furthermore, the effect of DBS of the shell of the NAcc was specific to cocaine seeking and did not influence food seeking.

The subthalamic nucleus was targeted in a study by Rouaud et al. (2010). Bilateral stimulation of the subthalamic nucleus reduced the motivation of rats to work for an injection of cocaine but increased motivation to work for sucrose. These authors also demonstrated a place preference effect of subthalamic nucleus DBS: preference for a place previously associated with the rewarding properties of cocaine was reduced, whereas preference for a place associated with food was increased.

Friedman et al. (2011) examined the effect of stimulation of the lateral habenula on sucrose reinforcing behavior. The lateral habenula was selected, because it is known to play a role in prediction of negative reinforcement, punishment, and aversive responses. Rats trained to self-administer 20% sucrose for 16 days were then stimulated in the lateral habenula. Stimulation significantly reduced sucrose self-administration levels. Also, an effect on conditioned place preference was found, leading the authors to conclude that lateral habenula stimulation attenuates positive reinforcement by natural substances.

3.5 Risks

DBS is an invasive procedure and as such carries the risk of transient or permanent complications such as infections, intracerebral hemorrhage, or failure of the device. A recent review of DBS for Parkinson disease which is the disease most often treated with this method has found the risk of intracerebral hemorrhage to be <2% (Bronstein et al. 2011), while some centers reported considerably lower incidences (University of Cologne: 0.2% in a series of 262 patients (Voges et al. 2006)). The total frequency of intraoperative complications was 4.2% in this latter series including transient (0.2%) or permanent (0.4%) neurological deficits. The late infection rate was 6.1%, and partial or complete removal of the system was required in 4.6% of the patients because of skin infection. During follow-up hardware-related problems occurred in 13.9% patients. There are also side effects of DBS on cognitive functions, behavior, and affect. However, these are specific to the stimulation site. The experience with DBS of the NAcc is still very limited. From the published case reports and small series it is difficult to derive a general impression of the spectrum of specific side effects. Transient hypomania has been observed (Heinze et al. 2009) and forgetfulness and word finding difficulties have been observed in some patients as a permanent side effect (Denys et al. 2010).

4 Available Evidence on DBS in Alcohol Dependence

The evidence for an effect of DBS in alcohol dependence is steadily growing but still rather weak. Controlled clinical trials with adequate numbers of patients, as they have been conducted for DBS in Parkinson's disease (Deuschl et al. 2006) and dystonia (Kupsch et al. 2006), for example, are still lacking.

An important case report was presented by Kuhn et al. (2007) who treated a 54-year-old patient with severe anxiety disorder complicated by secondary depressive disorder and alcohol dependence by bilateral DBS of the NAcc with the aim to alleviate anxiety and depression. Selection of the NAcc was based on previous experiences of these authors in a small series of patients receiving DBS of the NAcc for intractable obsessive–compulsive disorders (Sturm et al. 2003). They further argued that the NAcc is important for the pathophysiology of anxiety disorders, because of its central position within the amygdaloid complex, basal ganglia, mediodorsal thalamic nucleus, and prefrontal cortex. Whereas the patient experienced only a moderate reduction of his anxiety disorder and depression, he showed a remarkable reduction of alcohol intake such that one year after surgery he consumed alcohol only occasionally.

A small case series has been treated in Magdeburg, Germany (Heinze et al. 2009; Müller et al. 2009) with DBS of the NAcc. Three male patients participated in the study. One additional patient had a perioperative infection and therefore did not receive treatment with DBS. Patient HM (aged 36 at the time of the operation)

had started to drink alcohol at age 12 and had a family history of alcoholism (father and two uncles). Prior to the operation the daily dose of alcohol amounted to 2 to 30.7 l bottles of hard liquor per day. More than 60 inpatient detoxification treatments and three prolonged withdrawal therapies had been unsuccessful as well as treatment with acamprosate. The patient was implanted in October 2007 and has been abstinent since then (last follow-up 2011). There were no psychological changes after the operation. The patient reported to have no craving symptoms and that he is thus able to derive pleasure from daily activities of life. He has found a job and has established new social contacts. Patient GM (age 37 at the time of the operation) had started to drink alcohol at age 11 and had a positive family history (father, mother, several other relatives). His first detoxification treatment was at age 15 and he took part in three prolonged withdrawal therapies of 3 months' duration each. Acamprosate treatment had to be discontinued because of side effects. The longest period of abstinence prior to the operation had lasted 3 months during which the patient had experienced massive craving and intense reactions to alcohol-related cues. Following the operation (Jan 13, 2008) this patient experienced a period of hypomania which stopped after stimulation parameters were changed. The patient has been abstinent since the operation and reports a complete reduction of his reaction to alcohol-related cues and craving (last follow-up 2011).

Patient TM (age 40) had been alcohol-dependent since age 18 and a positive family history (father). Numerous withdrawal therapies had been unsuccessful. In 2005 he was sentenced to a jail sentence of 3 years during which he continued to drink. The preoperative MRI was unremarkable except for a small signal-intensity in the right temporal region, most likely a residual change after a contusion. The patient was operated on September 13, 2007 and showed no psychological abnormalities in the postoperative period. He was fully abstinent until September 2008. Subsequently, he has experienced short periods of relapse of 1–2 weeks duration (10 weeks in the past 16 months). The patient remarked that he had never felt as good as currently and reported a considerable reduction in his reaction to alcohol-related cues. These positive experiences led to the initiation of a clinical trial of DBS of NAcc sponsored by the Deutsche Forschungsgemeinschaft which will be conducted at the University of Magdeburg, Germany, with the Charité, Berlin, and the Medical School Hannover, Germany, as further participating institutions.

Kuhn et al. (2011) recently reported on an additional patient with severe alcohol addiction who was treated with DBS of the NAcc. One year after surgery, normalization of addictive behavior and craving was found.

Further evidence for an effect of DBS of the NAcc on addictive behaviors comes from observations of smoking behavior of patients. For example, Kuhn et al. (2009a) investigated smoking behavior in ten patients treated with DBS of NAcc for various disorders (Tourette's syndrome, obsessive–compulsive disorder, anxiety disorders) and found that three patients had quitted smoking without specific medical intervention. Interestingly, these patients also had been stimulated with a higher voltage than the rest of this small sample. The rate of spontaneous smoking cessation observed (30%) is considerably higher than what would be

expected in an untreated population ($\sim$10%). An additional 47-year-old female patient treated with DBS of the NAcc for chronic treatment-refractory obsessive–compulsive disorder and comorbid nicotine dependence and obesity has been reported by Mantione et al. (2010). Following surgery, unintended, effortless smoking cessation as well as weight loss have been observed. This is another piece of evidence pointing to the positive effects of DBS of the NAcc in addiction. A further case-report described bilateral DBS of the NAcc in a patient addicted to heroin (Zhou et al. 2011). This patient had been treated without success by numerous earlier interventions. This patient remained abstinent for the follow-up period of 6 years even though the stimulator was turned off after about 3 years. In addition, a great reduction of cigarette consumption was observed in this patient.

With regard to other potential targets, some circumstantial evidence has accumulated with regard to the subthalamic nucleus. There is a report on two patients with Parkinson's disease who suffered from dyskinesias, motor fluctuations, and dopamine dysregulation syndrome, who were treated with bilateral DBS of the STN (Witjas et al. 2005). Interestingly, in the first patient, a 38-year-old man with an 8-year history of PD, dopamine dysregulation syndrome and excessive alcohol intake, alcohol problems were greatly reduced after stimulation was initiated. In addition, dopaminergic medication could be discontinued. It is thus unclear whether the effect on alcohol consumption might, at least in part, be due to the change in dopaminergic medication. In the second patient, a 53-year-old man with a 5-year history of PD, dopaminergic treatment could be reduced by 75% after stimulation. In this case, it is unclear whether the amelioration of the motor symptoms was the main reason for the decreased need for dopaminergic medication or whether there was a direct effect of DBS reward circuits.

5 Deep Brain Stimulation as a Window into the Addicted Brain

While obviously not the main focus of DBS treatment of addiction, the application of this treatment opens up new possibilities for basic research into the cognitive neuroscience of addiction (Münte et al. 2007, 2008; Cohen et al. 2009a, b, c). First, most patients are awake during the implantation procedure for clinical reasons. Thus, it is feasible to record electrophysiological activity from the target structure during the operation while the patient is performing a cognitive task probing the function of the target structure. Intraoperative recordings are confined to a few minutes only, as the operation may not be prolonged for scientific reasons. Therefore, several groups have begun to record from externalized leads post-operatively, i.e., before the electrodes are connected with the stimulator.

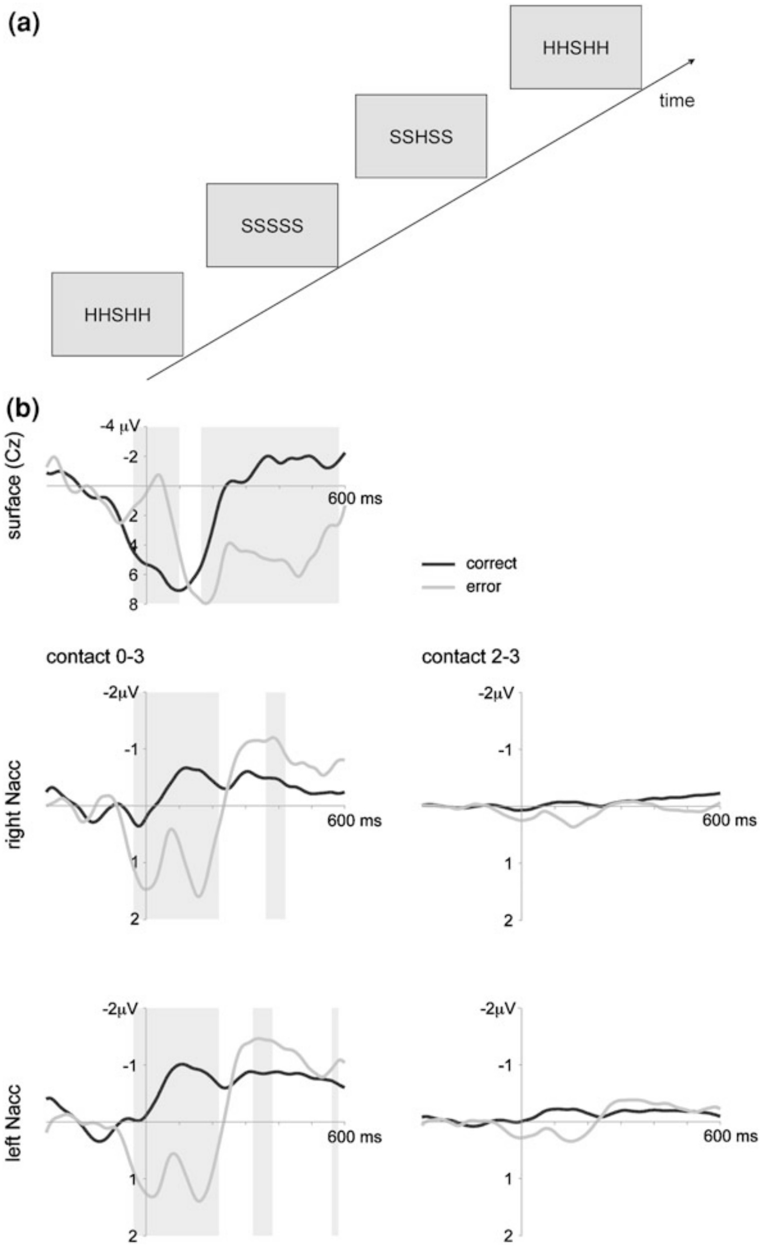
With regard to the most promising target structure for addictive disorders, the NAcc, a number of studies have recorded from this structure addressing different aspects of reward processing, action monitoring, and incentive salience addiction

(Münte et al. 2007, 2008; Cohen et al. 2009a, b, c; Heinze et al. 2009). Recordings were made not only in patients treated for addiction but also in OCD patients and depression, for whom the NAcc has also been proposed as a possible target. In this section we will give a few examples to illustrate how such research might inform theories of addiction.

A key feature of addictive behaviors is that they seem to be deeply engraved in the addict's brain resisting attempts to delete or alter them. The concept of 'wanting' [used in quotes as, for example, by Berridge and Robinson (2003); (Robinson and Berridge 2008)] has been proposed to explain important aspects of addictive behavior. It refers to an underlying implicit and objective motivation process, incentive salience, and can be dissociated from hedonic aspects of addiction (termed 'liking'). 'Wanting' (but not 'liking') leads to compulsive drug intake even in situations in which the addict does not expect to experience a positive affect. The incentive-sensitization theory of addiction proposed by Berridge and Robinson combines neural sensitization and incentive salience concepts. It has been found that following alcohol or drug intake dopamine is released in the NAcc (Koob and Le 1997, 2008; Everitt and Robbins 2005; Everitt et al. 2001) and this release is thought to attribute "incentive salience" to drug-associated cues (Robinson and Berridge 2008). This in turn may mediate 'wanting' of alcohol or other drugs of abuse. Furthermore, through the alcohol-induced stimulation of dopamine release, a down-regulation of striatal dopamine D2 receptors occurs which may underlie the neural sensitization (Volkow et al. 1996). Indeed, alcohol-addicted patients with down-regulated dopaminergic neurotransmission in the ventral striatum are at a higher risk for relapse (Dettling et al. 1995; Heinz et al. 1996; George et al. 1999). The question thus arises whether recording from the NAcc may allow to examine cue-related incentive salience.

A number of neuroimaging studies have found cue-related activations in the ventral striatum (Kilts et al. 2001, 2004; Myrick et al. 2008; Vollstädt-Klein et al. 2011). For example, Braus et al. (2001) presented alcohol-associated cues to alcohol-dependent and control participants during functional magnetic resonance imaging and observed activation of the ventral putamen in the former but not in the latter. Besides the putamen, drug-cue-induced craving has also been found to lead to activation of the perigenual and ventral anterior cingulate gyrus, the dorsolateral prefrontal and orbitofrontal cortex, insula, hippocampus, amygdala, and the ventral tegmental area (see Sinha and Li 2007, for a review). Previous studies of cue-related craving have either used active or passive viewing of the drug-related cue stimuli. Passive viewing has been suggested to be preferable to active viewing (Heinz et al. 2004) as rating or other cognitive tasks have been shown to reduce affective neural responses (Taylor et al. 2003). However, even passive viewing may engage active evaluation processes by the participant. We therefore decided to use a visual search task in which alcohol-related cues occurred outside the focus of attention. The task was adapted from previous studies addressing visual selection processes (Hopf et al. 2000; Woodman and Luck 1999).

We therefore conducted an experiment in which the alcohol-related cue stimuli not only was made task-irrelevant but was also shown outside of the focus of



attention of the participant. Participants viewed a series of stimuli each comprising an array of four pictures: one red, one green, and two grays. Colored and gray pictures were arranged in rows respectively and could occur in the upper or lower row. In any given run participants had to either attend to the red or the green

◀**Fig. 1** **a** Paradigm: participants have to react to the central letter (S or H) with either the *left* or *right* hand. The four flanking letters are irrelevant but induce action conflict and errors. They also lead to an increased need for action monitoring. **b** Averaged surface event-related potentials (electrode Cz referenced against mastoid process) and bipolar averaged LFPs obtained time-locked to the erroneous motor response. A typical error-related negativity followed by the so-called error positivity was seen in the ERP (significant differences, $p < 0.005$, as revealed by a bootstrapping procedure, between error and correct trials shaded in *grey*). In the NAcc on both sides similar error-related modulations were seen which were much more pronounced in the bipolar recordings between the two most distant contacts 0 and 3 than in the recordings between contacts 2 and 3. Activity from both sides was very similar

stimuli in order to indicate by speeded button press with the right index or middle finger whether the picture represented a "living" or "non-living" thing. The participants had to fixate fixation cross in the middle of the stimulus array during the entire run. The gray distracter pictures were always presented contralaterally to the target picture and could either be neutral or contain alcohol-related cues. These conditions effectively block out conscious perception of the distracter pictures. If any differential activity for trials with alcohol-related versus neutral distracter items would be seen under these conditions in the depth recordings, this would further support the notion of an automatic activation of the ventral striatum/NAcc by drug-related information. This is in fact what we found in two patients undergoing DBS for severe alcohol addiction. Averaged local field potentials (LFP) showed an early difference between the waveforms obtained to stimulus-arrays with a neutral distracter and those with an alcohol-related distracter. These results underscore the role of the NAcc in mediating incentive salience as it apparently was differentially modulated by stimuli that occurred outside of the focus of visual attention. This suggests that alcohol-related cues are processed in a highly automatic fashion.

In a number of studies, we (Heinze et al. 2009; Münte et al. 2007, 2008) have addressed action monitoring processes in patients receiving DBS of the NAcc either because of alcohol addiction or because of OCD. Human actions are not error-free, which is why error detection and correction are key cognitive processes. Both, electrophysiological and neuroimaging studies have provided evidence for a human action monitoring system. Importantly, response-locked event-related potentials (ERPs) feature a mediofrontal negativity in response to erroneous but not to correct button presses, termed the error-related negativity that is believed to be generated in the anterior cingulate cortex (Falkenstein et al. 1990; Luu and Tucker 2001; Gehring et al. 1993; Rodriguez-Fornells et al. 2002). The error-related negativity has been firmly established as a robust and reliable marker of error detection and has been used to assess changes in action monitoring in a number of neuropsychiatric diseases, such as depression, obsessive compulsive disease, or mediofrontal brain damage. Theoretical accounts have proposed that the error-related negativity is driven by reinforcement learning signals originating in the mesencephalic dopamine system (Holroyd and Coles 2002). This system modulates the activity of the anterior cingulate cortex. An error, detected by comparing an internal "efference copy" of an ongoing action with the action goal,

is thought to lead to a phasic decrease in dopamine release and thus to the elicitation of an ERN in the ACC. To elicit a high number of errors, flanker tasks are usually employed which comprise the rapid presentation of letter-strings (HHHHH, SSSSS, HHSHH, SSHSS) with the center letter requiring a button press with either the left hand (for letter S) or the right hand (for letter H). Importantly, incongruent flanker letters induce a high number of performance errors. Given that the midbrain dopaminergic system projects not only to the medial frontal cortex but also to NAcc which is richly innervated by dopaminergic input from the midbrain, it seemed reasonable to expect an error signal in the local field potential in the NAcc. This was in fact the case in a 39-year-old male patient with OCD who showed clear error-related modulations of the LFP which preceded surface activity by 40 ms (Münte et al. 2007). This initial finding was replicated in an alcohol-dependent patient from the Magdeburg series (patient GM, (Heinze et al. 2009)). Averaged LFP and surface ERP data are shown in Fig. 1.

In an elegant series of studies, Cohen and co-workers have used recordings from DBS electrodes placed in the NAcc because of depression and OCD to investigate different aspects of reward-related behavior (Cohen et al. 2009a, b, c; 2012). More research along these lines will hopefully be conducted as more patients will undergo DBS implantation for addiction.

6 Ethical Issues

The previous sections of this review have suggested that DBS may evolve into an effective treatment of addictive disorders. In addition to controlled clinical trials establishing the effectiveness of DBS in a sufficient number of patients, a number of ethical questions need to be addressed prior to its introduction as a routine treatment.

Unfortunately, DBS in psychiatric conditions such as addictive disorders is often viewed against the rather meek successes of psychiatric neurosurgery in the first half of the past century. Moreover, these early efforts in psychiatric neurosurgery have been carried out in badly selected patients, with no regulatory or ethical oversight, and for ambiguous indications (Lipsman et al. 2011). Carter and Hall (2011) have therefore proposed to restrict clinical trials to severe and intractable cases of addiction and to those patients that have the capacity to consent.

Deeper ethical and philosophical questions have been raised by other researchers. Insertion of stimulating electrodes may lead to cognitive, behavioral, and emotional disturbances (Wojtecki et al. 2011; Kirsch-Darrow et al. 2011) and also psychosocial changes (Smeding et al. 2011). It has been proposed that patients may attribute their behavior not to themselves but see it as produced by the stimulation electrode (Kuhn et al. 2009b). Thus, one could argue, that the therapeutic interference with the valuation of stimuli (including alcohol and drugs) and with goal-directed behavior via electrical stimulation of subcortical, non-conscious processes directly calls the autonomy of the treated patient into question. It is therefore mandatory to carefully evaluate the consequences of DBS treatment for perception, emotion, cognition,

and decision making. These ethical questions, while receiving increasing attention in the literature, pertain similarly to DBS in conditions other than addiction, however. Moreover, these concerns need to be weighed against the effects of alternative treatments such as psychotropic drugs. We do not see, why DBS in addictive disorders should be considered ethically more problematic than, say, DBS in dystonia.

7 Perspectives

DBS in addictive disorders is still in its infancy. Controlled clinical trials are greatly needed and are currently underway. These trials need to determine whether this costly treatment is warranted, whether it will even save money in the long run, what patients are suitable candidates for the procedure, and what are the long-term effects and efficacy of the treatment.

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