Physiol Rev 89: 649–705, 2009;
doi:10.1152/physrev.00013.2008.

Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior

RAINER SPANAGEL

Department of Psychopharmacology, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

I. Introduction 650
A. Alcohol use from an evolutionary and sociocultural perspective 650
B. The dark side of alcohol use and abuse 650
C. The pros of alcohol consumption 651
D. An integrative systems approach towards alcohol addiction 652
II. Primary Targets of Alcohol 653
A. Towards the identification of specific alcohol-sensitive sites on receptors and ion channels 653
B. Receptor composition determines sensitivity to ethanol 655
C. What are the functional consequences of the primary alcohol targets? 656
D. Drug discrimination to study the psychotropic effects of ethanol 656
III. Neurochemical Systems and Signaling Pathways Involved in the Action of Alcohol 657
A. The mesolimbic dopamine system and modulatory neurochemical systems: actions of alcohol 657
B. Acquisition of alcohol reinforcement is mediated by mesolimbic DA neurons 659
C. Are endogenous opioids and endocannabinoids involved in mediating the rewarding and pleasurable effects induced by alcohol? 662
D. Signaling pathways involved in alcohol reinforcement 663
IV. Gene Transcription and Epigenetic Effects Mediated by Alcohol 666
A. Gene transcription induced by ethanol 666
B. Epigenetic effects induced by ethanol 668
V. Synaptic and Cellular Effects Mediated by Alcohol 670
VI. Neuronal Network Effects Induced by Alcohol 671
A. Multielectrode recording to reveal neuronal network activity underlying alcohol-related behavior 671
B. Human brain imaging to identify the neuroanatomical and neurochemical substrates of addictive behavior 672
C. Animal brain imaging to identify the neuroanatomical and neurochemical substrates of addictive behavior 675
VII. Behavioral Effects Induced by Alcohol: From Controlled Drinking to Alcoholism 676
A. An animal model to study different phases of alcohol consumption 676
B. An animal model to study alcohol-seeking behavior 677
VIII. Comorbidity, Genetic, and Environmental Factors That Contribute to Alcohol Use and Addictive Behavior 678
A. Anxiety and alcohol drinking/addictive behavior 678
B. Depression and alcohol drinking/addictive behavior 679
C. Gene × environment interactions and alcohol drinking/addictive behavior 680
IX. Treatment Aspects 682
A. Preclinical medication developments for the treatment of craving and relapse 682
B. Translational approach in medication development and new clinical trials 685
C. Individualized pharmacotherapy for alcoholism 690
X. Summary and a Perspective of Systems-Oriented Alcohol Research 690
A. A retrospective view of neurobiological alcohol research 690
B. A summary of the present review 691
C. A perspective of systems-oriented alcohol research 692

Spanagel R. Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior. *Physiol Rev* 89: 649–705, 2009; doi:10.1152/physrev.00013.2008.—Alcohol consumption is an integral part of daily life in many societies. The benefits associated with the production, sale, and use of alcoholic beverages come at an enormous cost to these societies. The World Health Organization ranks alcohol as one of the primary causes of the global

burden of disease in industrialized countries. Alcohol-related diseases, especially alcoholism, are the result of cumulative responses to alcohol exposure, the genetic make-up of an individual, and the environmental perturbations over time. This complex gene × environment interaction, which has to be seen in a life-span perspective, leads to a large heterogeneity among alcohol-dependent patients, in terms of both the symptom dimensions and the severity of this disorder. Therefore, a reductionistic approach is not very practical if a better understanding of the pathological processes leading to an addictive behavior is to be achieved. Instead, a systems-oriented perspective in which the interactions and dynamics of all endogenous and environmental factors involved are centrally integrated, will lead to further progress in alcohol research. This review adheres to a systems biology perspective such that the interaction of alcohol with primary and secondary targets within the brain is described in relation to the behavioral consequences. As a result of the interaction of alcohol with these targets, alterations in gene expression and synaptic plasticity take place that lead to long-lasting alteration in neuronal network activity. As a subsequent consequence, alcohol-seeking responses ensue that can finally lead via complex environmental interactions to an addictive behavior.

I. INTRODUCTION

A. Alcohol Use From an Evolutionary and Sociocultural Perspective

A conventional evolutionary perspective is that psychoactive drug use in humans is a novel feature of our environment and of cultural developments (338). However, given the fact that the evolution of animals proceeded in a world rich in drugs, a novel theory favors the concept that drug and alcohol intake by mammals and other species has always been an everyday occurrence (123, 479).[1] Thus occasional and even chronic intake of alcohol through sugar-rich plant products susceptible to fermentation, such as nectar, sap, and fruit, might be a behavioral feature that has been shaped over millions of years from the fruit fly to numerous mammals including primates and humans. This current theory is best exemplified by a very recent discovery in a primary tropical rainforest in West Malaysia, where pentailed tree shrews (*Ptilocercus lowii*) consume intoxicating amounts of alcohol on a daily basis (531). Pentailed tree shrews are mammals closely resembling modern primates' early ancestors who lived more than 50 million years ago, and their major daily food source is the nectar from the bertam palm *Eugeissona tristis*. This indigenous plant bears flowers that actively produce, by means of a number of hitherto unknown yeast species, alcohol in concentrations up to 3.8%, which is comparable to that of beer. In this million-year-old ecosystem, the pentailed tree shrew has adapted to a daily intake of intoxicating amounts of alcohol, most probably by means of metabolic tolerance, without suffering from any obvious negative consequences (531). In conclusion, this new discovery favors the hypothesis that from an evolutionary perspective alcohol intake behavior has been shaped over millions of years and should be considered as being part of our normal behavioral repertoire, embedded today in traditional and sociocultural contexts.

The great majority of Western modern society regularly consumes alcohol. The main reasons for the consumption of alcohol are that it can produce positive mood states and has stress-relieving effects. Thus alcohol is a daily incentive and, in addition to coffee and tea, alcoholic beverages are the most important commodities worldwide. In fact, Europeans spend ~100 billion euros on alcoholic beverages annually, which is reflected by the high rate of alcohol consumption per capita of 10 liters of pure ethanol per year. Luxemburg has the highest level of consumption worldwide at more than 13 liters per year. In comparison, the alcohol consumption per capita in North America in the last decade averaged 8.5 liters per year (Fig. 1).

B. The Dark Side of Alcohol Use and Abuse

Consuming and abusing these huge amounts of alcohol clearly also has a dark side, with enormous health and socioeconomic impacts on the world population. Thus in 10–20% of consumers, chronic alcohol use and abuse contributes to a multiplicity of medical complications including damage to organs and immune functions. Although most body organs are affected by alcohol intoxication and chronic alcohol use, severe alcohol-induced diseases are most notable in the liver, pancreas, and brain. Alcohol-induced brain damage is a particular problem during pregnancy, resulting in fetal alcohol syndrome, which represents the most common form of acquired mental disability, affecting up to 7/1,000 infants (340). During adolescence, the consequences of alcohol drinking, especially of binge drinking, on organ dysfunction and damage are largely unknown despite the fact that by 2007 binge drinking among adolescents had reached a prevalence rate of ~30% in various European countries.

New research programs have been recently launched, in particular by the National Institute of Alcohol and Alcoholism (NIAAA), to gain a better understanding of binge

[1] The terms *alcohol* and *ethanol* are used interchangeably throughout this review. However, the term *ethanol* is mostly used in the context of a specific effect, e.g., a specific pharmacological effect.

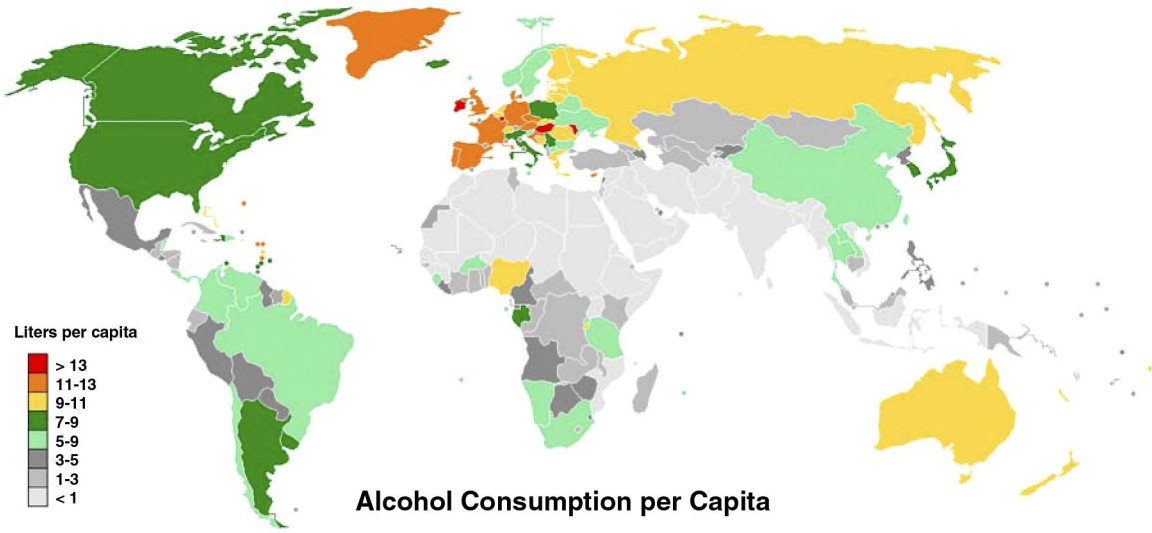


FIG. 1. Alcohol consumption per capita in liters of pure ethanol.

drinking during adolescence (www.niaaa.nih.gov). Initial results clearly indicate the negative consequences of such behavior. Thus the young adolescent brain displays higher sensitivity to alcohol-induced brain damage and cognitive impairment than the adult brain, in humans as well as in rodents (104, 449, 469, 529). Furthermore, the onset of alcohol use during adolescence leads to a higher susceptibility to stress-induced alcohol consumption (444, 147) and a greater risk of developing alcohol addiction in adulthood (167).

Alcohol use and abuse affects all social and ethnic groups; in almost every family in Western societies there will be someone who has suffered, directly or indirectly, from alcohol abuse. In an estimate of the factors responsible for the global burden of disease, alcohol contributes to 3.2% of all deaths worldwide (530). Moreover, with regard to the world population, the percentage of the total disability-adjusted life years (DALYs; calculated by adding the years of life lost due to premature mortality and the years of life lost due to living with disability) resulting from chronic alcohol abuse has been estimated to be as high as 4% (compared to 2.2% for AIDS). Alcohol use and abuse not only entails deleterious consequences to the physical and psychological health of the afflicted individuals (345), but also serious societal and economic fallout in the form of criminality, decreased productivity, and increased healthcare costs. As a consequence, on a worldwide scale, >10% of an industrialized nation's gross domestic product is spent in connection with alcohol use and abuse.

Alcohol abuse has a high comorbidity with other psychiatric disorders (238, 481). People who suffer from anxiety disorders and depression often use alcohol as a kind of self-medication (see sect. VII), but in most cases the driving force of alcohol abuse is the development of an addictive behavior. Addiction is defined as a syndrome in which alcohol or drug use pervades all life activities of the user.[2] Life becomes governed by the drug, and the addicted patient can lose social compatibility (e.g., loss of partner and friends, loss of job, crime). Behavioral characteristics of this syndrome include compulsive drug use, craving, and chronic relapses that can occur even after years of abstinence. The diagnostic criteria for alcohol addiction (in DSM-IV termed as alcohol dependence) according to this definition are listed in Table 1.

C. The Pros of Alcohol Consumption

Despite the enormous negative health and socioeconomic impact of alcohol use and abuse on the world population, light-to-moderate alcohol consumption also has several beneficial human health effects. These include reduced risk of coronary heart disease, type 2 diabetes, and some types of cancer (187). A substantial proportion of the benefit of moderate drinking is due to the pure ethanol component of alcoholic beverages; however, differences in the beneficial effects of various alcoholic beverages may occur (98). In particular, red wine contains a high number of polyphenols, such as resveratrol that can increase the function of the endogenous antioxidant system (27). Although research continues on resveratrol, the

[2] Note that the term *dependence* is avoided in this review. Addiction is a pathological behavioral syndrome that has to be strictly separated from physical dependence. Transient neuroadaptive processes underlie physical dependence to alcohol, whereas persistent changes within specific neuronal systems underlie addictive behavior. To avoid any confusion between clinicians, psychologists, and preclinicians, the term *dependence* should refer to a state of physical dependence.

TABLE 1. *Diagnostic guidelines: DSM-IV criteria for alcohol dependence*

Criteria for Alcohol Dependence
A definite diagnosis of alcohol addiction should be made by three or more of the following seven criteria, occurring at any time in the same 12-month period:
1. Tolerance
2. Withdrawal
3. Alcohol is often taken in larger amounts or over a longer period than was intended
4. There is a persistent desire or there are unsuccessful efforts to cut down or control alcohol use
5. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects
6. Important social, occupational, or recreational activities are given up or reduced because of alcohol use
7. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the alcohol (e.g., continued drinking despite recognition that an ulcer was made worse by alcohol consumption)

Diagnostic guidelines/criteria for alcohol dependence are from *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.) (DSM-IV). Washington, DC: American Psychiatric Association, 1994. Similar diagnostic guidelines have been developed by the World Health Organization (ICD-10). Note: DSM-IV is currently undergoing revision with publication of DSM-V planned in 2011. There is an ongoing discussion whether tolerance should be further included and whether a more quantitative measure such as the frequency of engaging in a harmful drinking pattern might not be a more practical approach for early diagnosis and intervention (207 and several commentaries in the same issue).

concentration in wine seems too low to account for the so-called French Paradox, which is the observation that the French suffer a relatively low incidence of coronary heart disease despite a diet relatively rich in saturated fats. Very recently, another group of polyphenols, known as procyanides, has been identified. Tests of 165 wines demonstrated that the greatest concentrations are found in European red wines from certain areas, which correlates with the longevity in those regions, such as southwestern France (99).

D. An Integrative Systems Approach Towards Alcohol Addiction

Taking into consideration all the pros and cons of alcohol and drug use, it is an ongoing challenge for all countries and governmental regulations to find a balanced way in which alcohol and other psychoactive drugs may be embedded into our daily life. In this context, it is important to have a solid understanding of how alcohol acts to induce its effects and, even more importantly, to understand the pathological mechanisms leading to addiction.

Over the last 20 years, great progress has been made in alcohol pharmacology. Today we have a solid understanding of how alcohol acts in the brain to induce its acute behavioral effects. Despite the generally held view that alcohol is an unspecific pharmacological agent, recent molecular pharmacology studies demonstrated that alcohol has only a few known primary targets. These are the *N*-methyl-D-aspartate (NMDA), γ-aminobutyric acid A ($GABA_A$), glycine, 5-hydroxytryptamine-3 ($5\text{-}HT_3$), and neuronal nicotinic acetylcholine (nACh) receptors, as well as L-type Ca^{2+} channels and G protein-activated inwardly rectifying K^+ channels (507). Following the first hit of alcohol on specific targets in the brain, a second wave of indirect effects on a variety of neurotransmitter/neuropeptide systems is initiated (507), leading to the typical acute behavioral effects of alcohol, ranging from disinhibition to sedation and even hypnosis, with increasing concentrations of alcohol.

It should be emphasized that alcohol can also exert a variety of actions and behavioral effects via its metabolic products. Thus acetaldehyde, which is the first product generated during alcohol metabolism, can affect the activity of different neurotransmitter systems and, subsequently, can contribute to the behavioral effects of alcohol (381). Nonoxidative alcohol metabolites, such as fatty acid ethyl esters, exert powerful effects on intracellular Ca^{2+} homeostasis (368) and therefore may also be important in mediating, at least in part, the actions of ethanol.

Multiple signaling pathways activated by alcohol and possibly by its metabolites lead to alterations in gene expression (114, 408). As a consequence of repeated alcohol intake, more or less long-lasting cellular and neurophysiological changes that trigger alcohol-seeking behavior become apparent in the brain reinforcement system. Whether or not this behavioral response transforms into an addictive behavior finally depends on the genetic make-up of an individual, as well as on numerous environmental factors (Fig. 2).

Addictive behavior is, therefore, the result of cumulative responses to alcohol exposure, the genetic make-up of an individual, and environmental perturbations over time. The complex gene × environment interaction leads to a large clinical heterogeneity, in terms of both the symptom dimensions and the severity of the disorder. Having highlighted this complex interaction, it is obvious that a reductionistic approach has certain limitations in achieving a better understanding of the pathological processes leading to an addictive behavior. Instead, a perspective of systems-oriented biomedicine, in which all

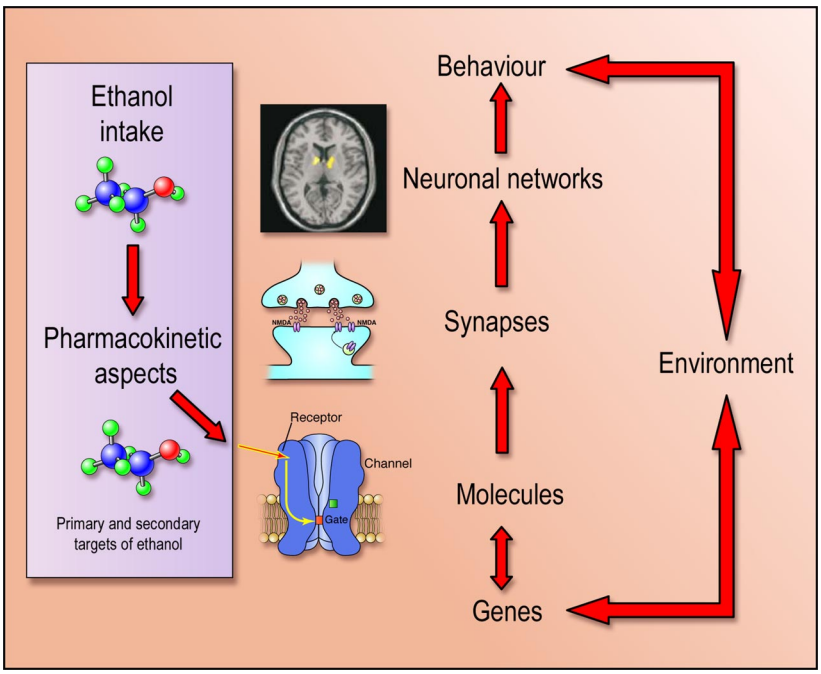


FIG. 2. This scheme shows a systems approach towards a better understanding of the acute and chronic effects of alcohol. This review follows exactly this approach. Thus sections II and III describe the primary and secondary targets of alcohol including signaling transduction. Section IV discusses effects on gene transcription along with epigenetic effects. Synaptic and cellular effects are summarized in section V. Section VI describes neuroimaging and anatomical work leading to an understanding of the neuronal networks underlying the action of alcohol. Finally, sections VII and VIII describe behavioral responses and their interaction with environmental effects such as stress. Note, although pharmacokinetics of ethanol also determine the behavioral response to acute and chronic ethanol exposure, this review does not focus on the pharmacokinetic aspects.

interactions and dynamics of all endogenous and environmental factors involved are centrally integrated (Fig. 2), is suggested to lead to further progress (5).

II. PRIMARY TARGETS OF ALCOHOL

How does alcohol affect the functions of the central nervous system (CNS)? It is only recently that a shift from the so-called lipid theory (the primary targets of ethanol are membrane lipids) to the protein theory (the primary targets of ethanol are membrane proteins, especially receptors) has taken place (363). Into the 1990s, different lipid theories postulated that alcohol acted via some perturbation of the membrane lipids of CNS neurons. In particular, effects on membrane fluidity and disordering of the bulk lipid phase of membranes were originally an attractive hypothesis of alcohol action because it provided a possible mechanism by which alcohol could affect membrane proteins, such as ion channels, via an action on membrane lipids.

There are, however, clear limitations to this hypothesis. First, the effects of alcohol on membrane disorder are generally measurable only at alcohol levels well above the pharmacological range [>500 mg/dl blood alcohol levels (BALs); these levels are close to the LD_{50} of ethanol in humans].[3] Significant effects of membrane disordering on protein function are even more difficult to envision at pharmacologically relevant alcohol concentrations. For example, at very high intoxicating BALs associated with loss of consciousness (~300 mg/dl), there would only be 1 alcohol molecule per ~200 lipid molecules (363). Second, membrane effects induced by alcohol concentrations exceeding the pharmacological range can be mimicked by an increase in temperature of just a few tenths of a degree Celsius (363), which clearly does not produce behavioral signs of alcohol intoxication or appreciably alter the function of membrane proteins such as neurotransmitter-gated ion channels. Therefore, the reported effects of alcohol on membrane fluidity and organization seem to be a purely biophysical phenomenon with no relevance to the pharmacological CNS effects of alcohol. Taking even more refinements of the lipid theory into consideration (363), it remains very unlikely that membrane lipids are the primary targets of alcohol.

A. Towards the Identification of Specific Alcohol-Sensitive Sites on Receptors and Ion Channels

The protein theory predicts that alcohol acts specifically on membrane proteins such as receptors and ion channels. The main reason for a shift towards the protein theory originates from findings that alcohol, at concentrations in the 10–20 mM range, directly interferes with the function of several ion channels and receptors.[4] In a key publication, David Lovinger et al. (283) demonstrated that

[3] For historical reasons, blood alcohol concentrations are calculated as g/kg blood plasma given in percent. Since the specific weight of plasma is 1.23, a BAL of 500 mg/dl corresponds to 4.06‰.

[4] For reference, a low intoxicating BAL of 50 mg/dl is equivalent to an ethanol concentration of 10.6 mM.

NMDA function was inhibited by ethanol in a concentration-dependent manner over the range of 5–50 mM, a range that also produces intoxication. The amplitude of the NMDA-activated current was reduced 61% by 50 mM ethanol. What is more, the potency for inhibition of the NMDA-activated current by several alcohols is linearly related to their intoxicating potency. This suggests that ethanol-induced inhibition of responses to NMDA receptor activation may contribute to the neural and cognitive impairments associated with intoxication (283). But how can ethanol directly interfere with NMDA receptor function?

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 some modulatory function on channel activity, especially under pathological conditions. Electrophysiological studies show that ethanol interacts with domains that influence channel activity (536), suggesting that residues within transmembrane (TM) domains may be involved. In the search for a possible binding site of alcohol at the NMDA receptor, several site-directed mutagenesis studies have been performed and putative binding sites in TM3 and -4 of the NR1 and NR2A subunits, respectively, identified (389, 390, 409, 450) (Fig. 3). In particular, a substitution of alanine for a phenylalanine residue in the TM3 of the NR1 subunit

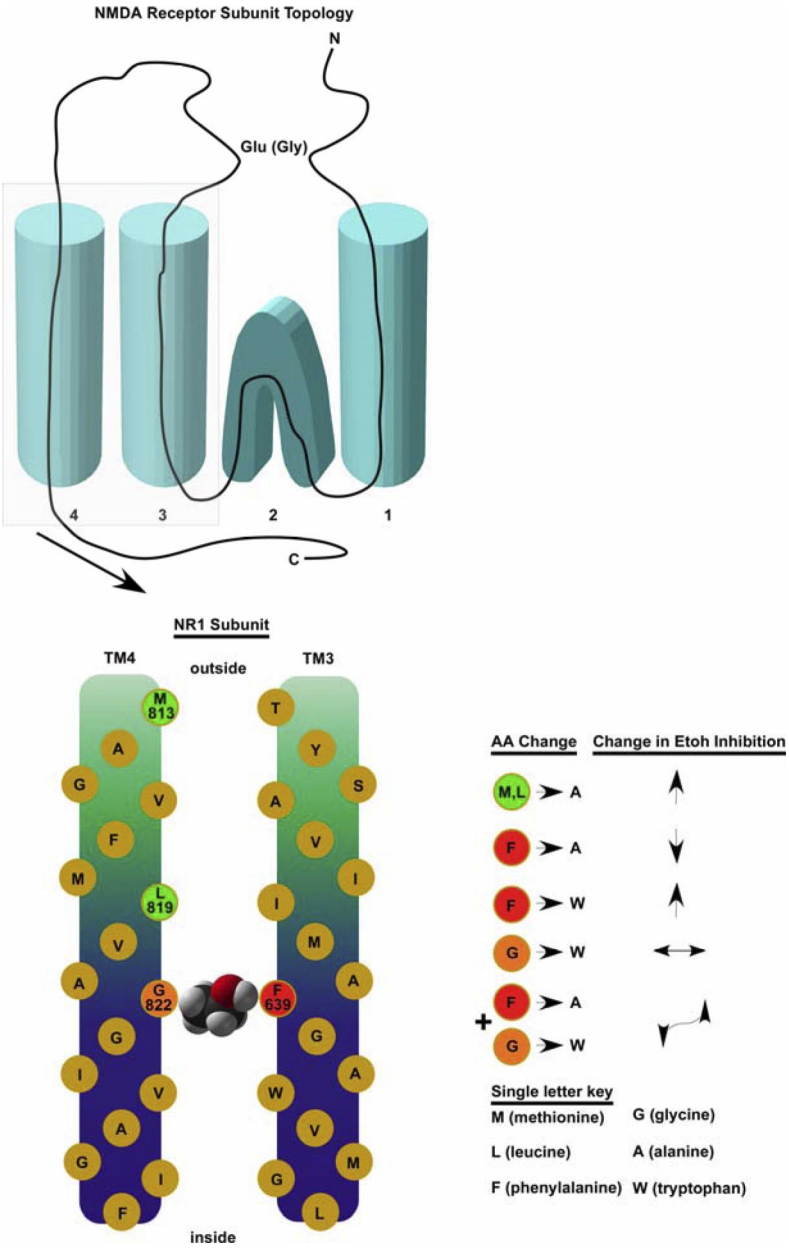


FIG. 3. Site-directed mutagenesis reveals sites of action of ethanol on the NMDA receptor. Exchanges on amino acids (AA) and their consequences on ethanol inhibition of NMDA currents are indicated. Residues in the TM3 and TM4 domains of the NR1 subunit were identified that either enhanced (green) or reduced (red) ethanol inhibition of NMDA currents. In particular, substitution of TM3 alanine for phenylalanine (F639A) strongly reduced ethanol inhibition, and this effect was reversed by replacing TM4 glycine with tryptophane (G822W). (Figure kindly provided by J. J. Woodward and C. T. Smothers.)

strongly reduced the ethanol sensitivity of recombinant NMDA receptors (409).

Besides the NMDA receptor, other receptors or ion channels expressed within the CNS also have putative alcohol-binding sites. In particular, the function of $GABA_A$ receptors is enhanced by ethanol. The $GABA_A$ receptor/chloride channel complex is a pentameric ligand-gated ion channel and the major inhibitory neurotransmitter receptor in the mammalian brain. Several subunits have been identified, with the majority of $GABA_A$ receptors being composed of α-, β-, γ-, and δ-subunits (23). With the use of different receptor constructs, a region in the TM domains of the α/β subunits of the $GABA_A$ receptor was identified which is involved in the action of ethanol (318), where it can potentially bind to a water-filled protein cavity between the second and third TM segments of these receptor subunits. In addition to its effects on $GABA_A$ receptors, ethanol also directly affects glycine receptors. Thus there is considerable evidence to indicate that ethanol acts on specific residues in the TM domains (318) as well as on the extracellular domain of glycine receptors, and the net effect on receptor function is the summation of positive and negative modulatory effects of ethanol on different ethanol-sensitive binding sites (103). Furthermore, ethanol potentiates neuronal nACh (336) and $5\text{-}HT_3$ receptor function (282, 289). The $5\text{-}HT_3$ receptor mediates fast synaptic transmission at postsynaptic sites and regulates neurotransmitter release presynaptically, and its alcohol sensitivity has been consistently shown in different in vitro preparations (308).

Non-ligand ion channels also constitute a primary target of ethanol. Thus ethanol inhibits dihydropyridine-sensitive L-type Ca^{2+} channels, and single-channel recordings suggest that the effects of ethanol on gating are consistent with the interaction of a single drug molecule with a single target site, possibly the L-channel itself (522). In addition, ethanol opens G protein-activated inwardly rectifying K^+ channels (GIRKs) (246, 269). Selective enhancement of GIRK2 function by intoxicating concentrations of ethanol was demonstrated for homomeric and heteromeric channels, and a region of 43 amino acids in the carboxy (COOH) terminus has been identified that is critical for the action of ethanol on these channels (246, 269).

B. Receptor Composition Determines Sensitivity to Ethanol

These primary inhibitory and facilitatory actions of ethanol on ion channels and receptors depend on a number of variables, in particular the ethanol concentration and the subunit composition of a particular channel or receptor. For example, ethanol's action on $GABA_A$ receptors strongly depends on the subunit composition. While most subunit compositions of $GABA_A$ receptors display responses to ethanol only at high concentrations (>60 mM), it has been found that very low concentrations (1–3 mM) of ethanol do alter the activity of $GABA_A$ receptors containing δ subunits. These GABA receptors are exclusively associated with $\alpha4/\alpha6$ subunits and the $\beta3$ subunit in vivo. Moreover, in $\alpha4\beta\delta$ subunit combinations, receptors containing the $\beta3$ subunit have been found to be almost 10 times more sensitive than receptors containing the $\beta2$ subunit, suggesting that the $\beta3$ subunit also constitutes an ethanol-sensitive site (519). However, mouse models in which either the $\beta3$ subunit was genetically deleted or knock-in mice that carry a single point mutation[5] in the β subunit do not differ in their acute response to ethanol when compared with wild-type animals (424). These findings suggest that "extrasynaptic" δ subunit-containing $GABA_A$ receptors (without a prominent role of the associated $\beta3$ subunit), but not their "synaptic" γ subunit-containing counterparts, are primary targets for ethanol.

The subunit composition of glycine receptors and other receptors is also critical in the response to ethanol. Thus $\alpha1$-containing glycine receptors appear to be more sensitive to low concentrations of ethanol than $\alpha2$-containing receptors (317). Furthermore, ethanol concentrations lower than 100 mM are known to potentiate only $\alpha2\beta4$, $\alpha4\beta4$, $\alpha2\beta2$, and $\alpha4\beta2$ subtypes of nACh receptors. In contrast, $\alpha3\beta2$ and $\alpha3\beta4$ subtypes are not affected by these ethanol concentrations, while $\alpha7$ receptor function is inhibited (178). Higher ethanol concentrations are less selective and potentiate almost all nACh receptors. As a result of the differential distribution of the aforementioned receptors as well as their subunits throughout the brain (e.g., $5\text{-}HT_3$ and neuronal nACh receptors are primarily expressed in the cerebral cortex and some limbic regions, while the NR1/NR2B subtype of NMDA receptor is primarily expressed in forebrain regions), ethanol affects some brain regions more than others.

It is not yet possible directly to measure by means of biophysical methods the binding of an ethanol molecule to these receptors or ion channels due to the fact that ethanol is a small molecule with low binding energy and is only efficient in the mid-millimolar range. These pharmacological characteristics preclude a direct assessment of an ethanol protein-binding site. However, with the discovery of the LUSH protein in the fruit fly *Drosophila melanogaster*, it became possible to model how TM residues can form a specific protein-binding pocket for ethanol. The high-resolution crystal structures of LUSH in complex with a series of short-chain alcohols were obtained by David Jones's team in 2003 (254). LUSH's struc-

[5] N265M: the in vivo action of general anesthetics is strongly attenuated by this point mutation (227).

ture reveals a specific alcohol-binding site. LUSH exists in a partially molten globule state. The presence of ethanol at pharmacologically relevant concentrations <50 mM shifts the conformational equilibrium to a more compact state (65), demonstrating that ethanol induces a conformational change of the binding protein, an important requirement for a functional binding site. A group of amino acids form a network of concerted hydrogen bonds between the protein, and the ethanol molecules provide a structural motif to increase alcohol-binding affinity at this site. This motif seems to be conserved in a number of mammalian ligand-gated ion channels, and it is therefore suggested that the alcohol-binding site in LUSH represents a general model for putative alcohol-binding sites in proteins such as the NMDA or $GABA_A$ receptors.

Finally, it should be noted that alcohol is an important odor signal in the sensory spectrum of fruit flies, and wild-type flies have an active olfactory avoidance mechanism to prevent attraction to concentrated alcohol whereas *lush* mutant flies are abnormally attracted to high concentrations of ethanol, propanol, and butanol but have normal chemosensory responses to other odorants (244). The ability of fruit flies to detect ethanol is important for chemotaxis towards food sources. However, adult flies are also susceptible to intoxication and death in high ethanol environments (76), in a range similar to that observed in humans, making them an ideal animal model for the study of alcohol intoxication (329). In conclusion, there is a selective advantage in the ability of fruit flies to avoid environments with dangerously high alcohol concentrations, and LUSH is required for this response.

C. What Are the Functional Consequences of the Primary Alcohol Targets?

Taken together, over the last 20 years it has been demonstrated that ethanol acts directly on membrane receptors and ion channels. This favors the protein theory, and the current view commonly held is that ethanol has only a few known primary targets that include NMDA, $GABA_A$, $5\text{-}HT_3$, and nACh receptors, as well as L-type Ca^{2+} channels and GIRKs, where concentrations as low as 1 mM produce alterations in the function of these receptors and ion channels.

Although more structural information about the putative alcohol-binding sites on proteins such as the NMDA receptor continues to be acquired, the functional impact of these binding sites is still to be discovered. Advances will only be achieved by novel knock-in models such as those already described for the $GABA_A$ receptor (227), in which the wild-type receptor subunits are replaced by those containing alcohol-insensitive or -hypersensitive sites. In the meantime, we have to be content with the use of either knockout mice or specific pharmacological interventions in combination with an appropriate behavioral test for acute alcohol intoxication. A commonly used procedure is the loss of righting reflex (LORR), a behavioral test that probes the relevance of a particular receptor in alcohol intoxication. In this test, either a rat or mouse is injected with a high dose of ethanol (3–4 g/kg intraperitoneally) and upon becoming ataxic is considered to have lost the righting reflex. The animal is then placed on its back and LORR duration is calculated as the time that elapses until the animal is able to right itself. Although the LORR provides a reliable measure of CNS sensitivity in response to alcohol, it can be only used for a behavioral readout of the effects of hypnotic alcohol concentrations of at least 50 mM, which corresponds to BALs above 250 mg/dl. However, as stated above, most of the putative membrane protein-binding sites for alcohol are sensitive to much lower concentrations of ethanol; thus how is it possible to investigate whether alcohol binding to these targets has any psychotropic effects?

D. Drug Discrimination to Study the Psychotropic Effects of Ethanol

Drug discrimination studies with ethanol as a training drug provide a valuable tool to study the psychotropic effects during alcohol exposure. Drug discrimination studies can be conducted in humans as well as in laboratory animals and have been used for more than 30 years to understand whether a specific binding site on a protein is mediating an ethanol-like interoceptive stimulus; the numerous studies are well archived under www.dd-database.org. During a discrimination test the experimentor asks: "Do you feel like having alcohol?" In fact, the discriminative ethanol stimulus very much corresponds to the subjective effects experienced by social drinkers and can already be detected by BALs of 30 mg/dl (214).

As shown in Figure 4, animals can be trained in an operant task to discriminate ethanol from saline and, subsequently, in a so-called substitution/generalization test, a specific pharmacological agent (e.g., an NMDA receptor blocker such as memantine or ketamine) is applied to test whether this compound produces an ethanol-like stimulus. It is important in animal drug discrimination studies that self-administered ethanol can substitute for investigator-administered ethanol, as this demonstrates that the psychotropic effects of self-administered ethanol are similar to those produced by investigator-administered ethanol (288). Moreover, healthy social drinkers undergoing a computer-assisted intravenous alcohol self-infusion paradigm experienced a similar alcohol effect as with drinking (549), suggesting that irrespective of the route of administration similar psychotropic effects of alcohol are achieved.

Substitution studies have shown that a complete substitution for ethanol is exerted by NMDA receptor antag-

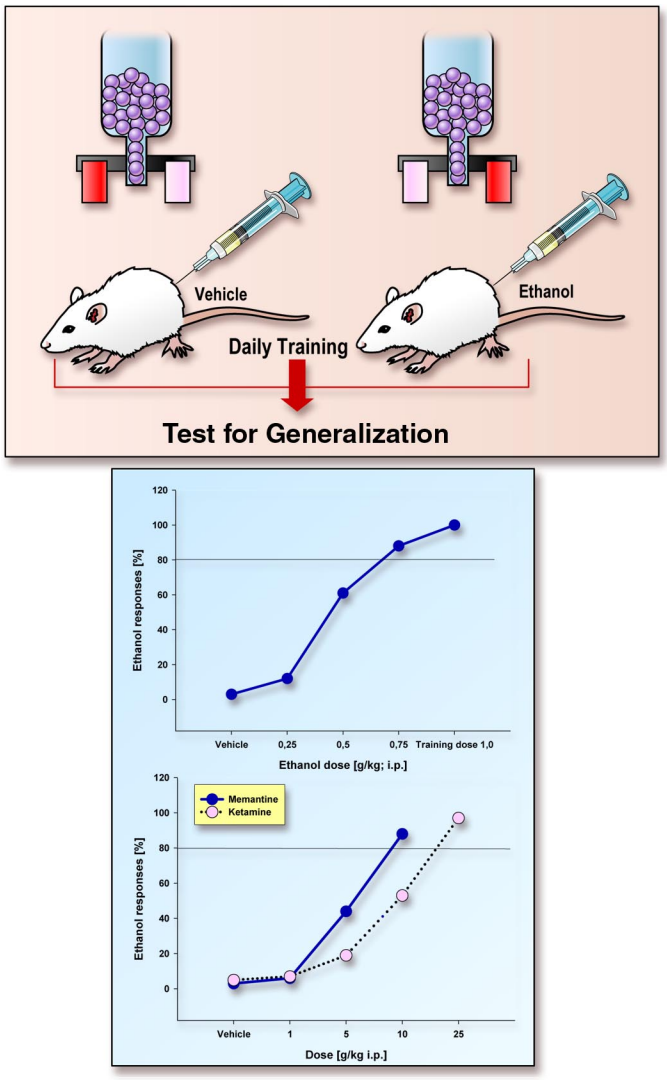


FIG. 4. Drug/ethanol discrimination is widely recognized as one of the major methods for studying the psychotropic effects of drugs. In drug discrimination studies, effects of drugs serve as discriminative stimuli that indicate how reinforcers (e.g., food pellets) can be obtained. For example, animals can be trained to press one of two levers to obtain food after receiving an ethanol injection (here the red active lever is on the right side, pressing the white lever has no consequences; 1.0 g/kg ip as training dose), and to press the other lever to obtain food after injection of vehicle (saline; here the red active lever is on the left side, pressing the white lever has no consequences). Once the discrimination has been learned, the animal will press the appropriate lever according to whether it has received ethanol or saline; accuracy in most experiments is very good (90% or more correct). Trained subjects can then be used *1*) to determine an ethanol dose-response curve (*left bottom panel*; note: a dose of 0.5 g/kg already produces 60% response accuracy, meaning that some animals already recognize the ethanol stimulus) and *2*) to determine whether a test substance (e.g., an NMDA receptor antagonist such as memantine or ketamine; *right bottom panel*) is identified as being like or unlike the ethanol training dose. This is the so-called generalization, or substitution, test (476).

onists and certain GABA-mimetic drugs acting through different sites within the $GABA_A$ receptor complex (193, 251). Thus it has been consistently shown in mice, rats, and monkeys that noncompetitive antagonists at the NMDA receptor, such as dizocilpine (MK-801), phencyclidine (PCP), ketamine, or memantine, which all act as an ion channel blocker, generalize to the ethanol cue while competitive NMDA antagonists have often shown only partial substitution for ethanol (92, 166, 198, 209, 443). Moreover, it has been demonstrated that ketamine produced dose-related ethanol-like subjective effects in detoxified alcoholics (255), suggesting that, at least in part, NMDA receptors mediate the subjective effects of ethanol in humans. Furthermore, the ethanol stimulus effect may be increased (i.e., stronger recognition) by drugs acting at nicotinic cholinergic receptors and $5\text{-}HT_3$ receptor agonists (251). Finally, depending on the training dose of ethanol, different receptors are involved in mediating the discriminative stimulus properties of the drug (165).

In conclusion, the ethanol stimulus is composed of several components, with the NMDA receptor and $GABA_A$ receptor complex being of particular importance. This demonstrates that the primary sites of alcohol's action do not simply induce intoxication but also mediate subjective effects. Therefore, an understanding of the receptor mechanisms that mediate the discriminative stimulus effects of alcohol can be used to develop medications aimed at decreasing the subjective effects induced by alcohol.

III. NEUROCHEMICAL SYSTEMS AND SIGNALING PATHWAYS INVOLVED IN THE ACTION OF ALCOHOL

The first hit of alcohol on specific targets in the brain leads to the typical acute subjective effects comprising the discriminative stimulus properties of this drug, and associated with these psychotropic effects, the intoxication signal ranging from disinhibition to sedation and even hypnosis occurs with increasing concentrations of alcohol. Following this first hit of alcohol, a second wave of indirect effects on a variety of neurotransmitter/neuropeptide systems is initiated (507); it is believed that this second wave, which mainly involves monoamines, opioids, and endocannabinoids, is crucial for the initiation of alcohol reinforcement and reward.

A. The Mesolimbic Dopamine System and Modulatory Neurochemical Systems: Actions of Alcohol

The brain regions that play an important role in mediating the reinforcing effects of drugs of abuse, including alcohol, have been identified by a variety of neuropharmacological studies that include lesion, microinjection, and microdialysis experiments. However, the groundbreaking work was performed in 1954 by Olds and Millner (347). Their electrical brain stimulation experiments made it apparent that the brain must have some special-

ized brain sites for reinforcement and reward functions. In these experiments brain sites were identified where electrical stimulation was rewarding in the sense that a rat will stimulate itself in these places frequently and regularly for long periods of time if permitted to do so (for an illustration of this technique, see Ref. 425). Drugs of abuse lead to an increase in sensitivity of the animal to the electrical stimulation. However, only oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation (328). The midbrain dopamine (DA) system, in particular, is sensitive to electrical self-stimulation and has been characterized as a neurochemical substrate of reinforcement (433, 533, 534). Midbrain A10 DA neurons involved in the initiation of reinforcement processes originate in the ventral tegmental area (VTA) and project to structures closely associated with the limbic system, most prominently the nucleus accumbens (NAC) shell region as well as the prefrontal cortex (PFC). Activation of the midbrain DA system by all kinds of reinforcers has been demonstrated in animals and humans. For example, by means of neuroimaging methods in humans (see sect. VI*B*), it has been shown that social attractiveness (230), sex and orgasm (155, 202), even classical music (but only in musicians; Ref. 51) can induce enhanced activity in the NAC. Also, a variety of drugs abused by humans, including alcohol, leads to enhanced mesolimbic DAergic activity, preferentially in the NAC shell region (115, 213, 379). In the following text, animal studies are described that examine the relationship between alcohol and midbrain DA.

Various techniques have indicated that the mesolimbic DAergic system is activated when alcohol is administered to laboratory animals. The VTA, in particular, has been implicated in the effects of alcohol. Thus, following the key publication by Gessa et al. (157), which showed that low systemic doses of ethanol produce a dose-dependent increase in the firing rate of DAergic neurons, later it was consistently shown that alcohol stimulates DA transmission in the mesolimbic pathway (115). With the use of microdialysis, it was found that acute administration of alcohol results in preferential release of DA from the NAC shell region (379). It is suggested that the manner by which acute alcohol administration increases extracellular DA within the NAC is via changes in GABAergic feedback into the VTA. Alcohol may decrease the activity of these GABAergic neurons, which subsequently leads to a disinhibition of mesolimbic DA neurons (467). This suggested mode of action is supported by the observation that DA levels within the NAC remained elevated after systemic alcohol administration, whereas somatodendritic release in the VTA had already declined, implying that alcohol also has local effects in the NAC (247). Since local infusion of a DA-reuptake inhibitor through the dialysis probe into the NAC elevated DA levels therein and, in parallel, decreased DA levels in the VTA (247), it is suggested that elevating DA levels in the NAC activates a long-loop negative GABAergic feedback system to the VTA, which regulates DA cell body neuronal activity (228, 247, 286, 467). In recent studies it has finally been demonstrated that the NAC is the primary hot spot for the DA releasing properties of ethanol but that a secondary effect occurs in the VTA as well (136, 278) (Fig. 5).

However, DAergic activity is regulated not only via a long-loop negative GABAergic feedback system and GABAergic interneurons within the VTA but also by a variety of other systems. Glutamatergic activity in particular also seems to control the mesolimbic DAergic pathway (148, 286). Glutamatergic projections from the PFC, bed nucleus of the stria terminalis, laterodorsal tegmental nucleus, and lateral hypothalamus feed into the VTA (350). In addition, glutamatergic projections from the PFC, hippocampus, amygdala, and paraventricular nucleus feed into the NAC, and glutamate release from any one of these projection terminals can act on ionotropic glutamate receptors in the NAC shell to induce DA release (44, 205, 361). In addition, glutamatergic neurons within the VTA have recently been identified (537), which might also influence DAergic activity via different glutamate receptors. Microdialysis studies have revealed biphasic effects of ethanol on glutamate release within the NAC. Thus, at low doses, ethanol may elevate extracellular glutamate levels in the NAC, whereas at higher doses it reduces glutamate overflow (148, 324). Whether this effect of alcohol on glutamatergic transmission within the mesolimbic DA system is of relevance for the activity of DA A10 neurons is less clear. For instance, infusion of an NMDA receptor antagonist into the VTA did not affect the

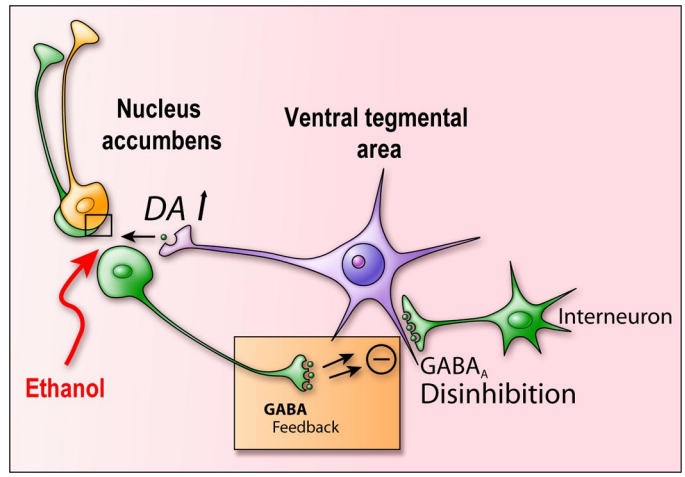


FIG. 5. Similar to all other drugs of abuse, ethanol stimulates dopamine (DA) release preferentially in the nucleus accumbens (NAC) shell region, and it is suggested that this neurochemical event is involved in the initiation of alcohol reinforcement. Although multiple neurotransmitter and neuropeptide systems are involved in the initiation of this neurochemical event, the disinhibition of GABAergic neurons appears to be one major contributory mechanism. [Modified from Spanagel and Weiss (467).]

DA-enhancing effects of ethanol (135). This is surprising in light of the fact that several other drugs of abuse act via glutamatergic input on the activity of midbrain DA neurons (148, 234) and, as such, clearly requires further research.

The dorsal raphe nucleus 5-HT system also modulates the DAergic activity of the VTA and the NAC (333). This 5-HT effect is mainly mediated via the 5-HT_3 receptor (284). Blockade of 5-HT_3 receptors therefore selectively prevents both ethanol-induced DA release in the NAC (71) and the somatodentritic release of DA in the VTA (69), whereas activation of 5-HT_3 receptors increases DA release within the VTA of Wistar and alcohol-preferring P rats (275). 5-HT_3 receptor-mediated effects on DA release may be due to a mixed primary action of ethanol on this receptor and a secondary effect of ethanol-induced serotonin release.

Neuronal nACh receptors are also a primary target of ethanol and are known to modulate the release of DA. The nACh receptor antagonist mecamylamine given systemically blocks the DA-releasing properties of ethanol (49). Furthermore, blockade of nAch receptors within the VTA also inhibits the stimulating effects of conditioned ethanol cues on DA neurons (279). This suggests that the nAch receptor-mediated acetylcholine/DA interaction may represent an important neurochemical access point of conditioned alcohol reinforcement. Moreover, this neurochemical interaction points to the synergistic effects of alcohol and nicotine in terms of reinforcement processes and provides a neurochemical correlate for the fact that alcohol drinking is strongly associated with smoking (272).

There also seems to be an interesting link between the acetylcholine/DA interaction and neuropeptides involved in feeding behavior such as ghrelin. Centrally administered ghrelin has DA-stimulating properties (218, 219) which appear to be mediated via central nAch receptors, suggesting that ghrelin activates cholinergic input into DA neurons. There is cholinergic input from the laterodorsal tegmental area to the VTA, and growth hormone secretagogue receptors (GHSR-1A), the functional ghrelin receptor, are expressed in both areas (219). It has been demonstrated that local administration of ghrelin into the VTA or the laterodorsal tegmental area enhances DA release in the NAC (219), suggesting that ghrelin may stimulate the mesolimbic DAergic system via activation of GHSR-1A in the VTA and laterodorsal tegmental area. Although a direct link between ethanol, ghrelin, and DA has not yet been investigated, it is known that ghrelin regulates not only energy balance and feeding behavior but is also likely to be directly involved in drug (105, 487) and alcohol reinforcement (428). It is currently unknown whether other neuropeptides involved in feeding behavior also modulate the action of ethanol on DAergic neurons. Such neuropeptides may include orexin A and B, which are synthesized exclusively in neurons of the lateral hypothalamus (417) and are activated in response to natural and drug reinforcers (176) including alcohol (262, 428). In addition, stimuli conditioned to alcohol availability also activate hypothalamic orexin neurons (110). Since there is a lateral hypothalamic orexin projection to both the VTA (139) and the NAC (21), it is probable that ethanol has an access point to the mesolimbic reinforcement system via these neuropeptides.

Finally, glycine receptors also modulate the DA release properties of A10 neurons since they are a primary target of ethanol. Thus reversed microdialysis of the competitive glycine receptor antagonist strychnine into the NAC decreases accumbal extracellular DA levels, whereas reversed microdialysis of the agonist glycine increases DA levels in the NAC (326). Furthermore, local perfusion of strychnine not only decreases accumbal DA levels per se, but also completely prevents an increase in accumbal DA levels following administration of ethanol (327).

In summary, systemic alcohol has multiple actions affecting the NAC, the VTA, and their afferents, i.e., there are multiple neurochemical points of access to DAergic A10 neurons. Most of these neurochemical access points represent primary targets of alcohol. Note that the activity of A10 neurons is also modulated by endocannabinoids and endogenous opioid systems (these modulatory mechanisms will be discussed in section III*C*). However, the most important questions remain unanswered: *1*) What are the behavioral consequences of the activation and modulation of DAergic A10 neurons by alcohol, and *2*) are alcohol reinforcement and reward and conditioned responses closely linked to DAergic activity?

B. Acquisition of Alcohol Reinforcement Is Mediated by Mesolimbic DA Neurons

Alcohol-induced activation of mesolimbic A10 neurons appears to be associated with the reinforcing properties of alcohol, since rats will directly self-administer alcohol into the VTA (149). In a more detailed study, Rodd et al. (402) demonstrated that rats will self-administer ethanol directly into the posterior but not into the anterior VTA. Coadministration of the DA D2/3 agonist quinpirole into the VTA at a concentration that activates DA D2 autoreceptors and thereby reduces the firing rates of VTA DA neurons was shown to prevent the acquisition of self-administration behavior into the posterior VTA. This effect was restored by the withdrawal of quinpirole or the infusion of the DA D2 antagonist sulpiride into the VTA (402). The results of this study indicate that alcohol is reinforcing within the posterior VTA and suggest that activation of VTA DA neurons is involved in this process (402).

Numerous pharmacological studies have further investigated the role of midbrain DA in alcohol reinforcement, but the results have been conflicting. Although 6-hydroxy-DA-induced lesions do not affect the maintenance of alcohol self-administration (212, 241, 287, 386), they substantially reduce the acquisition of alcohol drinking (212, 386). These findings indicate that the acquisition and maintenance of primary alcohol reinforcement may be mediated by different neuronal mechanisms and that functional midbrain DA neurons are not necessarily required to maintain alcohol self-administration. However, postsynaptic changes in DA receptor signaling appear to be involved in the maintenance of voluntary alcohol intake since DA D1 and D2 receptor knockout mice display altered alcohol consumption (102). In particular, operant alcohol self-administration behavior is markedly reduced in DA D2 receptor-deficient mice (373, 396). Quantitative trait locus (QTL) analysis using recombinant inbred mouse strains localized a QTL for alcohol preference at the location of the DA D2 receptor on mouse chromsome 9 (484). Furthermore, D1, D2, and D3 receptor agonists and antagonists are capable of modulating ethanol consumption in common stock rats (91, 369, 412) as well as in alcohol-preferring rats (125, 307, 489).[6]

DA measurements in different alcohol-preferring rat strains have also produced conflicting results. Alcohol self-administration has been shown to produce a considerably greater relative stimulation of mesolimbic DA release in alcohol-preferring P-rats than in control Wistar rats (31, 231, 524). In contrast to these findings, a similar dose-dependent increase in mesolimbic DA release in Finish alcohol-preferring AA rats and corresponding alcohol-avoiding ANA rats (454) has been reported by Kiianmaa et al. (242). Furthermore, in a well-designed experiment by the same authors (343), a group of AA rats drank 10% ethanol voluntarily in a limited access paradigm while a yoked group of AA rats and a yoked group of ANA rats received the same amount of ethanol intragastrically by intubation. Subsequently, the different animal groups underwent in vivo microdialysis. Then, DA release was monitored in the NAC after intraperitoneal challenge of 1 g/kg ethanol. The AA and the ANA rats that received ethanol noncontingently exhibited the same DAergic response to the ethanol challenge as naive animals in the previous experiment (242). The group of AA rats that had ingested the ethanol voluntarily even showed a significantly smaller increase in DA after the ethanol challenge (343). The latter result implies that tolerance develops to the DA releasing effect of ethanol in voluntarily drinking AA rats. This suggestion is further supported by yet another experiment in which DA release in the NAC was measured before and during alcohol drinking in AA rats. Self-administration of the ethanol solution had only a minor effect on DA levels during the first 10 min after the onset of drinking (344). Giving the rats a cue for ethanol, which was part of their daily, routine drinking regime, did not raise DA levels before ethanol was presented to the rats (i.e., during "anticipation") (344). Together, this consistent set of findings shows that mesolimbic DA is not the central substrate that produces the reinforcement from ethanol in AA rats.

Similar findings were obtained in a further line of alcohol-preferring rats. In alcohol-naive, high alcohol-drinking (HAD) and low-alcohol-drinking (LAD) lines of rats, alcohol dose-response curves for DA release exhibited no difference in the sensitivity to alcohol between the lines (354, 543). In a further comparative study, alcohol-naive HAD/LAD and AA/ANA rats were examined for their basal and ethanol-stimulated release of DA in the NAC by means of "no-net-flux" quantitative microdialysis. After completion of the neurochemical tests, the rats' voluntary alcohol intake and preference in the home cage were tested for 1 mo (233). Analysis of the data across individual animals and different lines revealed that extracellular DA and the percent of baseline increase in DA due to ethanol were significant predictors of ethanol preference (233).

With regard to the apparent lack of congruity among the aforementioned studies of DA release, the fact that most of these experiments were done with experimenter-administered alcohol must be taken into consideration, as this may explain why no differences are observed between the preferring and nonpreferring AA/ANA and HAD/LAD lines. Further studies are clearly warranted in rat lines where DA measurements are performed at a high-time resolution during operant self-administration. However, since the nonpreferring lines hardly respond to ethanol, appropriate experimental controls are lacking. The comparative study by Katner and Weiss (233), however, suggests that elevated extracellular levels of DA within the NAC and a greater responsiveness to enhancements in DA release by ethanol may be factors that contribute to high-alcohol preference. Furthermore, the data suggest that alcohol may be more reinforcing in animals that exhibit an enhanced DAergic response to initial ethanol exposure and, as such, may subsequently be associated with the acquisition of higher ethanol intake and preference.

The role of DA in mediating alcohol reinforcement has also been studied in the human brain. In an initial report by Ahlenius et al. (4), it was shown that α-methyl-*p*-tyrosine, a compound that blocks DA synthesis, de-

[6] Various alcohol-preferring and nonpreferring rat lines have been developed within the last 50 yr. Depending on the line, preferring rats consume 5–9 $g \cdot kg^{-1} \cdot day^{-1}$ ethanol, whereas the nonpreferring lines consume less than 1 $g \cdot kg^{-1} \cdot day^{-1}$. These lines are very powerful animal models in the study of the neurochemical substrates of alcohol reinforcement. A comprehensive overview of the different lines has recently been reported (31, 83, 93, 382, 354, 454).

creases ethanol-induced psychostimulation in humans. Using positron emission tomography (PET) measurements, Boileau et al. (60) demonstrated a significant reduction in [^{11}C]raclopride binding in the NAC in healthy volunteers after alcohol ingestion. In this study the magnitude of the change in [^{11}C]raclopride binding correlated with the psychostimulant effects of alcohol. This indicates that enhanced DA release occurs in response to alcohol drinking and that the degree of psychostimulation is mediated, at least in part, by augmented extracellular DA levels.

Given that DA plays a crucial role in the acquisition of alcohol reinforcement in animals and humans, it may be postulated that neurochemical points of access directly modulating DAergic activity (e.g., GABA, glutamate, serotonin, acetylcholine, glycine) must also play a crucial role in the acquisition of alcohol reinforcement.

$GABA_A$ receptors also play an important role in alcohol reinforcement, being both a primary target for alcohol and a direct neurochemical access point into the mesolimbic DAergic system. For instance, pharmacological manipulations of $GABA_A$ receptors with negative allosteric modulators were shown to reduce alcohol consumption in several alcohol-preferring rat lines (386, 523). In addition, antagonism of $GABA_A$ receptors within the VTA (342) or an increase in the activity of $GABA_A$ receptors in NAC (225) suppressed alcohol consumption in alcohol-preferring P-rats, suggesting the particular importance of $GABA_A$ receptors in both nuclei in alcohol reinforcement. Also, knockout mice lacking various $GABA_A$ receptor subunits were examined in several alcohol-related paradigms, and it was shown that α1, α2, α5, and δ subunit deletion leads to reduced alcohol consumption (53, 102, 226, 316). Furthermore, Sardinian alcohol nonpreferring rats, selected for their low alcohol preference and consumption (93), as well as ANA rats, carry a point mutation (R100Q) in the gene coding for the $GABA_A$ receptor α6 subunit, suggesting that the lack or malfunction of this subunit also contributes to reduced alcohol intake (74, 416).

The results of pharmacological studies using glutamate receptor antagonists in alcohol self-administration paradigms are less conclusive. Different NMDA receptor antagonists applied either systemically or locally into the NAC may reduce or have no effect on alcohol intake (40, 385, 443). The application of the AMPA/kainate receptor antagonist GYKI 52468 did not selectively alter operant response to alcohol (472). Neither did experiments with knockout mice suggest the involvement of AMPA receptors in the maintenance of alcohol drinking, as GluR1 and GluR3 deletions had no effect on either home-cage alcohol drinking or operant self-administration (101, 423). These more or less negative behavioral results do reflect the observations made at the neurochemical level. Thus, as previously mentioned, a clear modulatory role of glutamatergic input on DAergic A10 neuronal activity has so far not been established.

The dorsal raphe nucleus 5-HT system modulates the DAergic activity of the VTA and the NAC (333). This 5-HT effect is mainly mediated via the 5-HT_3 receptor (284). Blockade of 5-HT_3 receptors, therefore, selectively prevents both ethanol-induced DA release in the NAC (71) and the somatodentritic release of DA in the VTA (69). 5-HT_3 receptor-mediated effects on DA release may be due to a mixed primary action of ethanol on this receptor and a secondary effect of ethanol-induced serotonin release.

Knockout mouse models and pharmacological manipulations of various components of the 5-HT system have indicated a modulatory role for 5-HT in voluntary alcohol consumption. Deletion of 5-HT transporters (235) or overexpression of 5-HT_3 receptors (132) leads to a reduction in alcohol self-administration compared with that observed in control mice. Pharmacological manipulations of 5-HT system activity revealed that administration of a variety of serotonergic compounds were capable of reducing alcohol consumption in common stock as well as alcohol-preferring animals (263, 354, 545). 5-HT_3 receptor antagonists were shown to suppress the acquisition of voluntary alcohol consumption in alcohol-preferring P-rats. Furthermore, the reinforcing effects of ethanol within the posterior VTA of rats require activation of local 5-HT_3 receptors (403); a pattern therefore evolves linking the action of 5-HT_3 receptors on DAergic neurons within the VTA with alcohol reinforcement.

It has been shown that alcohol-induced stimulation of DAergic A10 neurons also involves central nACh and strychnine-sensitive glycine receptors, suggesting a possible involvement of these receptors in alcohol reinforcement. Infusion of mecamylamine into the VTA reduces voluntary alcohol consumption (134); however, it remains to be established which particular nACh receptor subunit composition is most important in this respect. It is known that α4β2 and α7 subtypes of nACh receptors do not play an important role in alcohol consumption (135, 265), whereas antagonism of α3β2 and β3 subunits of the nACh receptors has been shown to reduce voluntary alcohol consumption in both rats and mice (218, 260). Modulation of the activity of the glycinergic system also leads to reduced voluntary alcohol consumption. Molander et al. (235) have recently shown that the glycine reuptake inhibitor Org 25935, acting specifically on the glycine transporter 1, decreases alcohol preference and intake in rats by increasing extracellular glycine levels, which primarily activate inhibitory strychnine-sensitive glycine receptors. The picture that emerges once more highlights the importance of cholinergic and glycinergic input onto DAergic neurons in alcohol reinforcement.

In summary, animal research has demonstrated that midbrain DA A10 neurons and several modulatory neuro-

chemical access points, including $GABA_A$, $5\text{-}HT_3$, nACh, and glycine receptors, play an essential role in the acquisition of primary alcohol reinforcement processes. Thus mesolimbic DA activation is a property of ethanol and may possibly mediate its reinforcing effects. However, it must be emphasized that primary reinforcement processes do not necessarily reflect the emotional hedonic components of ethanol reward; it seems more probable that an enhanced DA signal highlights important stimuli and functions as a neurochemical learning signal for reinforcing stimuli (433, 467). Whether DA also plays a role in mediating hedonic aspects of alcohol intake is not known. However, the endocannabinoid and endogenous opioid systems may well serve as neurochemical substrates involved in the mediation of these positive mood states.

C. Are Endogenous Opioids and Endocannabinoids Involved in Mediating the Rewarding and Pleasurable Effects Induced by Alcohol?

Accumulating evidence indicates a central role for the endocannabinoid system in the regulation of the rewarding properties of drugs of abuse including alcohol (291). This system participates in drug reward through the release of endocannabinoids in the VTA. However, endocannabinoids are also involved in the motivation to seek drugs via DA-independent mechanisms (291), and an endocannabinoid hypothesis of drug reward has been postulated as an alternative to the DA hypothesis of drug reward. Endocannabinoids mediate retrograde signaling in neuronal tissues by the presynaptic cannabinoid (CB) receptors and are thus involved in the regulation of synaptic transmission by suppressing classical transmitter action. This powerful modulatory action on synaptic transmission has significant functional implications and interacts with the effects of drugs of abuse including alcohol. The endocannabinoid system includes CB1, CB2, and the orphan receptor GPR55 as a new CB receptor (261), endocannabinoids, e.g., 2-arachidonyl-glycerol (2-AG) and anandamide, their biosynthetic and inactivating enzymes and, perhaps, transporters for endocannabinoids (146).

Alcohol reinforcement processes are dependent on CB1 receptor activity. Thus CB1 receptors in alcohol-avoiding DBA/2 mice exhibit a lower efficacy than CB1 receptors in alcohol-preferring C57BL/6 mice (210). Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats or AA rat lines exhibit specific differences in the organization of the brain endocannabinoid system in a number of brain regions when compared with unselected Wistars or alcohol-avoiding ANA rats (86, 171), and CB1 receptor antagonism has been reported specifically to suppress acquisition of alcohol-drinking behavior in rodents (96). In general, pharmacological manipulation of the CB1 receptor influences ethanol intake and preference (15, 94, 158). Similarly, CB1 receptor knockout mice display reduced alcohol self-administration (488, 521). The study of Wang et al. (521) further demonstrated that there is an age-dependent decline in ethanol preference and intake in wild-type but not in CB1 knockout mice, which is consistent with reward-dependent mechanisms becoming less important with age and that a decrease of activity within the endocannabinoid system might correlate with these events. A direct link between alcohol reinforcement and alterations in brain endocannabinoid formation has recently been established. Alcohol self-administration was shown significantly to increase microdialysate 2-AG levels within the NAC, and the relative change in dialysate 2-AG content was significantly correlated with the quantity of alcohol consumed (67).

In summary, the endocannabinoid system is involved in DA-dependent reinforcement processes, but it also elicits DA-independent effects on reward. Whether these effects are associated with a pleasurable hedonic state induced by alcohol is not as yet known. CB1 receptor stimulation in humans can produce euphoric effects. However, it is of key importance to test whether administration of a selective CB1 receptor antagonist in volunteers, drinking small but stimulatory amounts of alcohol, will blunt the euphoric stimulatory effects of alcohol.

Such an alcohol challenge experiment has been conducted in social drinkers using naltrexone, an opioid receptor antagonist, to test whether the endogenous opioid system mediates subjective euphoric effects (120). Using a double-blind design, subjects received naltrexone or placebo and 1 h later consumed a beverage containing ethanol (0.5 g/kg). Breath alcohol levels were measured over 3 h after the beverage was consumed, and subjects completed standardized subjective effects questionnaires at regular intervals. Ethanol under placebo produced its prototypic effects, including positive subjective responses such as euphoria and increased ratings of overall liking. Surprisingly, pretreatment with naltrexone did not alter the positive subjective or any other effects of ethanol (120). The same experiment was repeated in light drinkers and moderate drinkers with the same outcome: naltrexone pretreatment had no dampening effect on the subjective response to ethanol (121). The situation is, however, quite different in heavy-drinking subjects; it has been repeatedly shown that naltrexone decreases subjective (e.g., liking) and psychomotor responses to alcohol in heavy drinkers (122, 309, 388).

It has long been suspected that endogenous opioid peptides, such as endorphins and enkephalins, are the neurochemical substrates of reward processes and are important for mediating the associated euphoric effects. Early studies showed that both enkephalins and endorphins possess intrinsic rewarding properties and are self-

administered by rodents directly into the brain ventricles (33, 505) and the NAC (162). The VTA is a further hot spot for opioids to induce reward, since opioid receptor agonists produce conditioned place preference when administered into this brain site (189) and are also self-administered into the VTA (111). Thus μ/δ opioid receptors, the targets of enkephalins and endorphins, in the VTA and NAC appear to be critically involved in the neurobiological mechanisms underlying reward (458). It has further been demonstrated that basal DA levels within the NAC are modulated by endogenous opioid systems (459). For many years, however, it was unclear whether drugs of abuse do, in fact, trigger reward-related processes via release of endorphins and enkephalins. In a key publication by Olive et al. (348), it was finally demonstrated by in vivo microdialysis that drugs of abuse, including ethanol, release endorphin into the NAC. Importantly, concomitant measurement of DA levels demonstrated that after administration of alcohol, the increase in extracellular levels of DA appeared to occur at an earlier time point than in the case of endorphin. This suggests that alcohol stimulates DA and endorphin in the NAC, but probably does so via independent mechanisms (299). Given the findings of studies showing the positive reinforcing properties of μ/δ agonists when injected into this brain region (162, 504), it is hypothesized that this increase in extracellular endorphin levels may play a role in the rewarding properties of ethanol and other drugs of abuse. The NAC receives endorphinergic input from pro-opiomelanocortin (POMC)-containing neurons in the arcuate nucleus of the hypothalamus (52, 145). It is unclear, however, whether the ethanol-induced increase in extracellular NAC endorphin levels is a result of direct activation of the arcuate-NAC endorphin pathway, as some studies have demonstrated that acute ethanol administration increases POMC mRNA in the arcuate nucleus (290, 383) while others have been unable to find any effect of acute ethanol on arcuate POMC mRNA content (245).

Importantly, the opioid receptor antagonist naltrexone reverses alcohol-induced DA release in the NAC in rats, and suppression of operant alcohol-reinforced behavior by naltrexone is associated with attenuation of the alcohol-induced increase in dialysate DA levels in the NAC (164). These findings not only show that alcohol reinforcement depends on the activity of endogenous opioid systems but also confirm that DA output in the NAC is associated with this reinforcement process (189). Furthermore, alcohol-preferring AA rats show lower opioidergic activity in areas involved in alcohol reinforcement (346), and many other studies have also reported innate differences in opioid systems in other alcohol-preferring and alcohol-avoiding lines of animals (189, 507). In addition, μ-opioid receptor knockout mice do not self-administer alcohol under several different test conditions (399) and, in accordance, selective antagonists acting at μ-opioid receptors are able to reduce alcohol consumption (211).

In conclusion, animal research clearly indicates that endocannabinoids and endogenous opioids play a crucial role in alcohol reward.[7] This further demonstrates interactions with the mesolimbic DA system as well as DA-independent processes. Owing to the limitation in animal studies that subjective states cannot be measured in an adequate way renders the translation of this knowledge to the human context difficult, and an understanding of how the subjective euphoric and hedonic aspects of rewards such as ethanol evolve in humans remains elusive. It may be speculated that a state of well-being and euphoria involves far more complex processes than merely the central activation of CB1 and μ/δ-opioid receptors, being likely to involve the whole body system, including a balance within the stress system and physiological parameters driven by the autonomic nervous system. In this respect, the hypothalamus, which interfaces the brain-body axis, may prove to be of importance.

D. Signaling Pathways Involved in Alcohol Reinforcement

In view of the role of DA in the acquisitition of alcohol reinforcement, over the past two decades various research groups have investigated signal transduction within the NAC and other areas receiving input from A10 neurons (114, 408). Following the release of DA, various DA receptors become activated. The D1-like receptors, which include DA D1 and D5 receptors, enhance the activity of adenylyl cyclase (AC) via coupling to stimulatory G proteins ($G\alpha_s$). Alternatively, D2-like receptors (D2-D4) inhibit AC through inhibitory $G\alpha_i$. D1-like receptor stimulation results in an increase in the concentration of cAMP and the activation of cAMP-dependent protein kinase A (PKA) signaling, which then leads to substrate phosphorylation. One of the substrates of PKA is the transcription factor cAMP response element-binding protein (CREB), which eventually results in increased transcription of genes containing cAMP response elements (CRE) in their promoter region (280). The cAMP-PKA pathway is a primary signaling cascade induced by exposure to alcohol (114, 408), and the expression of numerous ethanol-responsive genes is regulated by PKA (see sect. IV) (Fig. 6). Voluntary alcohol intake significantly decreases the expression of Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) and CREB phosphorylation, specifically in the shell of NAC (322), suggesting that decreased CaMKIV-dependent CREB phosphorylation in

[7] In addition, a functional cross-talk between the endocannabinoid and opioid systems has been found in the mutual modulation of drug/alcohol reinforcement and reward processes (143, 401).

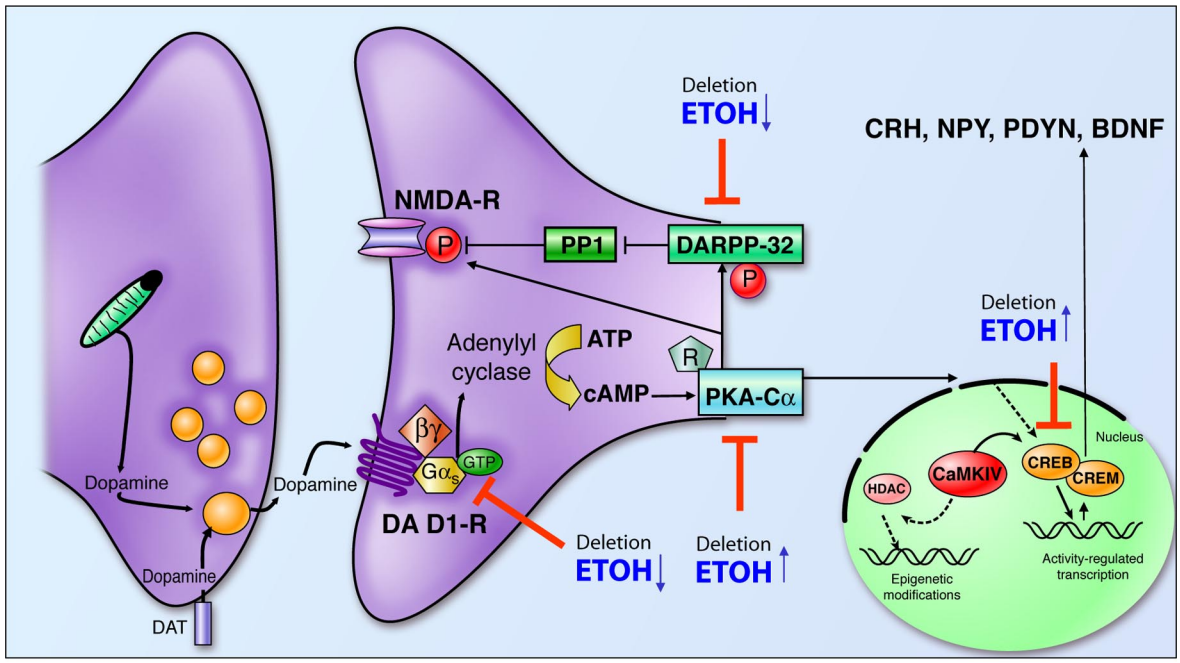


FIG. 6. Following the release of dopamine (DA) induced by ethanol, the DA D1 receptor is stimulated. Subsequently, the activity of adenylyl cyclase (AC), through coupling to stimulatory G proteins ($G\alpha_s$), results in an increase in cAMP concentration and in the activation of cAMP-dependent protein kinase A (PKA) signaling. cAMP induces this activation by promoting the dissociation of the regulatory subunit (R) of PKA from the catalytic subunit (PKA-Cα). PKA-Cα then leads to phosphorylation of the transcription factor cAMP response element-binding protein (CREB). Exposure to ethanol also influences the expression of Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) and thereby CREB phosphorylation in the NAC. These events finally result in altered transcription of genes containing a cAMP response element (CRE) in their promoter region such as corticotrophin-releasing hormone (CRH), neuropeptide Y (NPY), prodynorphin (PDYN), and brain-derived neurotropic factor (BDNF). Not only is CREB phosphorylated upon activitvation of D1 cAMP-PKA signaling but also DARPP-32, which is a 32-kDa protein that is expressed predominantly in striatal medium spiny neurons. In its phosphorylated form, it acts as a potent inhibitor of protein phosphatase 1 (PP1). The function of PP1 is the dephosphorylation of the NR1 subunit of the NMDA receptor. Therefore, PP1 inhibition by DARPP-32 leads to augmented NMDA receptor phosphorylation, which then increases channel function and counteracts the acute inhibitory action of ethanol on this receptor. Deletion or pharmacological blockade of $G\alpha_s$, $\beta\gamma$, PKA, or DARPP-32 leads to alterations in alcohol (ETOH) self-administration as indicated by the arrows. Note there are inconsistencies between the different knockout models and their alcohol consumption patterns; thus a reduction in cAMP-PKA signaling can lead to both reduced and enhanced alcohol consumption. These discrepancies are difficult to interpret and are not discussed in the relevant literature.

the shell region of NAC is involved in alcohol reinforcement. While the main role of CaMKIV may be activation of CREB, it has also been reported to regulate histone deacetylase (HDAC) trafficking (497). Interestingly, alcohol decreases HDAC activity and increases acetylation of histones (357) (Fig. 6 and sect. IV*B*).

The importance of cAMP-PKA signaling has been demonstrated in mice with genetically modified $G\alpha_s$ function. Mice lacking one $G\alpha_s$ allele exhibit low AC activity in the NAC and show decreased voluntary alcohol consumption compared with their wild-type littermates (520). Similarly, viral delivery into the NAC of a dominant-negative peptide that inhibits the $\beta\gamma$ subunits of G proteins reduces self-administration of alcohol in rats (539). These data imply that a reduction in cAMP-PKA signaling leads to reduced alcohol consumption. Surprisingly, however, augmented voluntary alcohol consumption is seen in knockout mice that lack a regulatory subunit of PKA (491). These mice also show a reduction in cAMP-stimulated PKA activity in the NAC and the amygdala. In line with this genetic manipulation of PKA activity, infusion of a PKA inhibitor into the NAC shell significantly increases voluntary alcohol consumption (321). Further PKA inhibition was shown to lead to decreased protein levels of the α-catalytic subunit of PKA (PKA-Cα) and phospho-CREB, indicating that decreased PKA/CREB function is involved in high alcohol preference (321). Indeed, innate high alcohol preference and excessive alcohol consumption, occurring for example in P-rats (31), is associated with lower phospho-CREB levels within the central amygdala (CeA) compared with NP rats. Infusion of a PKA activator into the CeA increased CREB function and decreased the alcohol intake of P-rats, whereas infusion of a PKA inhibitor into the CeA reversed the phenotype of NP rats with enhanced alcohol consumption and decreased CREB function (358). These results indicate that decreased CREB function in the CeA may be involved in the high alcohol consumption of P rats. In agreement with this is the finding that heterozygous CREB knockout mice also show enhanced alcohol consumption (358), although it remains questionable whether the latter finding is conclusive since the loss of CREB is readily compensated by

overexpression of CREM (208, 503), another member of the CREB family. In summary, regardless of the inconsistencies between the different knockout models and their alcohol consumption patterns, these data provide compelling evidence that PKA signaling modulates alcohol reinforcement processes and that reduced CREB function is seen after chronic alcohol exposure. In this context, a fundamental difference in alcohol-related cAMP-PKA signaling compared with other drugs of abuse should be emphasized, which is that an upregulation of CREB function is usually observed following chronic exposure to drugs such as cocaine (72, 408).

In addition to CREB, DARPP-32, a 32-kDa protein expressed predominantly in striatal medium spiny neurons, is also phosphorylated upon activation of D1 cAMP-PKA signaling. In its phosphorylated form, it acts as a potent inhibitor of protein phosphatase-1 (PP1) and, as such, is an important regulator of DAergic signaling (168). The function of PP1 is the dephosphorylation of the NR1 subunit of the NMDA receptor. PP1 inhibition by DARPP-32 therefore leads to augmented NMDA receptor phosphorylation, which then increases channel function and counteracts the acute inhibitory action of ethanol on this receptor (292). It should be emphasized that this enhancement of NMDA receptor activity in response to ethanol occurs only in dopaminoceptive neurons that contain D1 receptors along with the DARPP-32/PP1 cascade. This casacade may therefore play a critical role in synaptic plasticity induced by alcohol exposure, as DARPP-32-mediated enhancement of NMDA receptor function in striatal areas is likely to be an important factor in NMDA-dependent long-term potentiation (LTP), as outlined in section v. As a result of these cellular changes, DARPP-32 should be involved in the regulation of alcohol reinforcement. In fact, DARPP-32 knockouts voluntarily drink less alcohol than their wild-type littermates (397) (Fig. 6).

As well as cAMP-PKA signaling, early cell culture studies implicated the protein kinase C (PKC) pathway in the mediation of both acute and chronic responses to ethanol exposure (114, 339). PKC is a family of kinases that is activated by Ca^{2+}. Various PKC isoforms have been found in the brain. Following activation, they translocate to their substrate sites where they bind to scaffolding proteins, i.e., proteins that enable kinases efficiently to couple to specific targets such as receptors or ion channels. Important examples of scaffolding proteins involved in the actions and neuroadaptations of alcohol are Homer (482), RACK1 (502), and β-arrestin 2 (43). The two isoforms PKC-ε and PKC-ε interact with these scaffolding proteins, and they seem to be of particular importance in mediating alcohol-induced behavioral responses. PKC-γ knockout mice show enhanced alcohol preference (62) compared with wild-type mice, whereas PKC-ε knockouts exhibit a markedly reduced preference for alcohol (192). The latter phenotype could be rescued by means of inducible expression of PKC-ε in the NAC, and other forebrain areas restored alcohol preference in adult PKC-ε knockout mice to the level seen in wild-type mice (81). These findings indicate that PKC-ε signaling in the adult brain regulates alcohol reinforcement. Both PKCs seem to physically interact via phosphorylation with $GABA_A$ receptors in an opposing manner (339), resulting in reduced enhancement of $GABA_A$ receptor function by ethanol in PKC-γ knockout mice (177) or augmented function in PKC-ε knockouts (192).

As well as $GABA_A$, another key player in mediating the effects of alcohol is the glutamate receptor. The glutamatergic system is strongly linked to the intra- and extracellular messenger nitric oxide (NO) (63). Thus stimulation of NMDA receptors leads to Ca^{2+} influx, and binding of Ca^{2+} to calmodulin activates, among others, neuronal NO synthase which produces NO from arginine. NO is one of the few known gaseous signaling molecules and can act as a retrograde messenger. Activation of guanylyl cyclase and the resulting elevation of cGMP is a major downstream signal of NO in neurons. The full details of signaling through cGMP have not yet been clarified. cGMP affects several ion channels and phosphodiesterases in vivo. In many cells, the target of cGMP is the cGMP-dependent protein kinase I or II, abbreviated as cGKI and cGKII, respectively (200). In brain, NO, cGMP, and cGKII are closely related because both enzymes, neuronal NO synthase (nNOS) and cGKII, are frequently coexpressed, either directly or indirectly with cGKII-expressing neurons, which receive afferents from nNOS-containing neurons (200).

Evidence from pharmacological and knockout studies has implicated nNOS/NO/cGMP/cGKII signaling in the action of alcohol (Fig. 7); hence, administrations of compounds that inhibit all isoforms of NOS influence alcohol consumption in alcohol-preferring rats (68, 392). More importantly, nNOS knockout mice consumed six times more alcohol from high concentrated alcohol solutions than did wild-type mice (466).

In conclusion, NO signaling is critically involved in the regulation of alcohol reinforcement. Moreover, since nNOS knockout mice exhibit pronounced aggressive behavior (337), which was even augmented following alcohol treatment in an intermale aggression test (Spanagel, unpublished results), the close association of aggressiveness and alcohol drinking might also be related to alterations in the nNOS gene. In this respect, it should be realized that in humans aggressive personality is often associated with alcoholism (215) and, vice versa, alcohol consumption is associated with a high incidence of many different types of aggressive and violent behavior (376). Finally, the downstream components of NO in neurons, cGMP and its kinase, are also mediating some of the

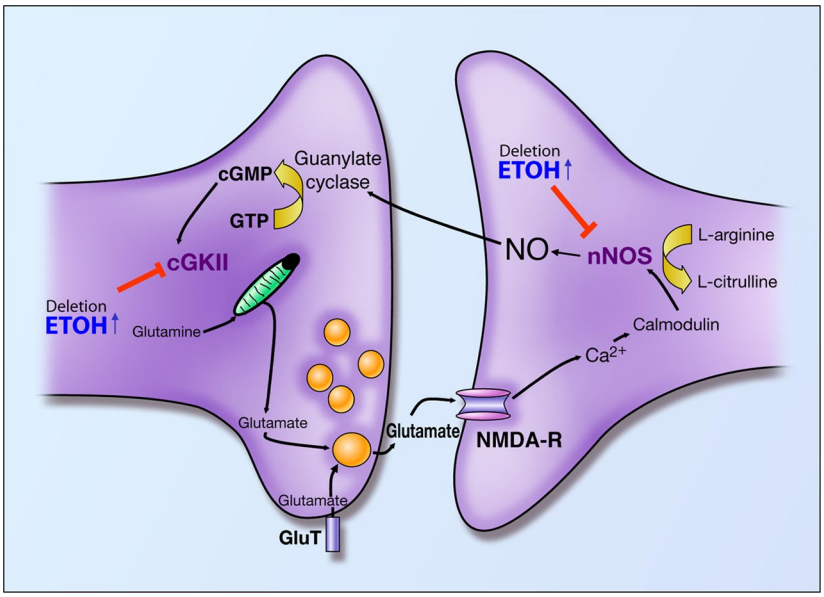


FIG. 7. Neuronal nitric oxide synthase (nNOS)/NO/cGMP/cGMP-dependent protein kinase II (cGKII) signaling is involved in mediating alcohol reinforcement. The stimulation of NMDA receptors leads to Ca^{2+} influx, and binding of Ca^{2+} to calmodulin activates nNOS which produces NO from arginine. NO acts as a retrograde messenger. The activation of the guanylyl cyclase and the resulting elevation of cGMP is a major downstream signal of NO in neurons. In neurons, the target of cGMP is the cGKII. Genetic deletion of nNOS and cGKII, respectively, leads to enhanced alcohol (ETOH) self-administration.

behavioral responses to alcohol exposure. Thus cGKII knockout mice voluntarily consume more alcohol compared with wild-type littermates (527). Overall, similarities of behavioral responses in nNOS and cGMPII knockouts suggest that the NO/cGMP/cGKII signaling pathway is involved in controlling alcohol reinforcement and other behavioral effects such as alcohol-induced aggressiveness.

In summary, cAMP-PKA signaling is involved in mediating effects of alcohol as well as influencing CREB-mediated processes. This altered CREB function affects multiple alcohol-responsive target genes that will be reviewed in section IV. In addition, cAMP-PKA signaling in medium spiny neurons affects DARPP-32 function which is, in turn, an important regulator of NMDA receptor function within the reinforcement system and may play an important role in neuroadaptations in response to alcohol exposure. NMDA receptors are closely linked to NO/cGMP signaling, and this pathway also plays a critical role in mediating alcohol reinforcement as well as other behavioral responses induced by alcohol. Finally, PKC signaling is also strongly affected by alcohol which, in turn, affects $GABA_A$ receptor function. Hence, alcohol affects the functioning of receptors (NMDA and $GABA_A$) relevant to synaptic plasticity (see sect. V) via various signaling pathways.

IV. GENE TRANSCRIPTION AND EPIGENETIC EFFECTS MEDIATED BY ALCOHOL

A. Gene Transcription Induced by Ethanol

The list of putative CREB target genes with CRE sequences now exceeds 100 and includes genes that control neurotransmission, cell structure, signal transduction, transcription, and metabolism (280). Given that several acute and chronic effects of ethanol are mediated by CREB, it can be assumed that CREB target genes are involved in mediating behavioral responses to ethanol. In fact, this has been demonstrated by pharmacological intervention studies and appropriate knockout models for a variety of CREB target genes, the most prominent being corticotrophin-releasing hormone (CRH) (181), prodynorphin (45), brain-derived neurotrophic factor (BDNF) (311), neuropeptide Y (NPY) (490), and numerous other genes (102). However, there are also many CREB-independent genes that may respond to alcohol, and the question is how can novel alcohol-responsive target genes and their products be identified in a hypothesis-free approach? Using the new -omics technologies, molecular expression profiles can be assembled and quantified on the mRNA, protein, and metabolite levels. In particular, there have been great advances in transcriptomics where expression levels of mRNAs in a given brain area or cell population are studied by one of the many gene expression profiling approaches (150). In particular, DNA microarrays are more and more applied as high-throughput technologies in alcohol research (151, 237).

Mammalian genomes are extensively transcribed but not necessarily translated (41), and this excessive RNA production may be an important contribution to the flow of information in a cell (475). Particularly, in the CNS, the site of RNA production can be some distance from the actual translation into proteins. Apart from cell bodies, substantial amounts of mRNA transcripts and other noncoding RNA species are found in different microregions of the neurons (e.g., dendritic spines, synaptic boutons), ready for activity-dependent translation, modulation by RNA editing, and degradation (380). Aware of the fact that

transcriptional changes do not reflect altered protein function, this section explores evidence for specific ethanol action on gene expression.

Similar to its neurochemical actions, effects of ethanol on gene expression can be seen, on a much slower time scale, as waves of subsequent events that depend on and interact with each other. Importantly, genomic effects are primarily found in those regions that are associated with the behavioral response. Site-specific effects within the structural and cellular complexity of the brain are a hallmark of pharmacological specificity of drug action. Stimulus-activated transcription of immediate-early response genes such as c-*fos* is a commonly used experimental paradigm to identify relevant brain circuits and cell types for drug action and even allows classification of drugs according to their neurochemical mechanism of action (480). Ethanol-evoked c-*fos* responses have been studied widely, and the specific activation patterns probably reflect action via several neurotransmitter systems (413). In fact, acute challenge with a moderate dose (1.5 g/kg ip) in drug-naive rats induces c-*fos* expression in brain regions associated with both rewarding and stressful ethanol actions (173). An alternative approach used transgenic mice carrying the reporter gene *lac*Z under the control of CRE. With the use of histochemistry to map CRE-mediated gene transcription in the brain of CRE-*lac*Z transgenic mice following ethanol injection, stimulus-activated transcription can be detected. Similar to the c-*fos* studies, LacZ staining upon an acute ethanol (1.5 g/kg ip) challenge was predominantly found in mesolimbic areas and brain regions associated with rewarding and addictive responses (16). This approach also suggests that cAMP/PKA signaling plays an important role in mediating ethanol effects on gene expression.

On the basis of the detailed mapping and knowledge of the brain circuitry involved in ethanol action, a growing number of studies have attempted a pharmacogenomic analysis of alcohol-responsive genes in the brain of experimental animals and humans. Since this has recently been comprehensively reviewed (201, 453), only a few results will be highlighted here.

Two main experimental strategies can be distinguished to study the genomic effects of ethanol on the brain. One type of study employed a variety of paradigms of acute or chronic ethanol challenge to analyze expression profiles during various periods of acute or protracted withdrawal that lasted from a few hours to several weeks (100, 108, 236, 394, 453, 500, 509). Alternatively, alcohol-responsive genes can be found by comparing the gene expression patterns of drug-naive rats that are selectively bred for differences in ethanol preference, because selected alleles underlying the behavioral response are also expected, at least in part, to mediate the pharmacological response to the drug (14, 38, 42, 83, 405, 452, 454). Although these studies are all highly variable in terms of experimental conditions (e.g., ethanol dose, route of administration, duration of exposure, time of sample collection, brain area of interest, behavioral consequences, animal lines, and various parameters concerning the microarray platform), the resulting lists of differentially expressed genes display some striking similarities regarding the biological themes that may be involved in the action of ethanol. The transcriptional response to ethanol seems to be related to two major functional groups: neuroplasticity and metabolism. Nearly all studies point to few, distinct signaling pathways and a wide range of differences in metabolic pathways.

As well as these common changes throughout all studies, the brain area of interest is a major determinant for particular pathways and individual genes that are affected by ethanol. An example of region-specific ethanol effects is the dysregulation of myelination-associated genes in the prefrontal cortex which is observed in both animal and human postmortem studies (144, 236, 237, 305, 453). Myelin-related genes play a role in axon remodeling, and the prefrontal cortex seems highly sensitive to the toxic effects of ethanol. Another case of region-specific ethanol effects is the upregulation of glia-derived angiotensinogen seen in the prefrontal cortex of chronically ethanol-exposed rats as well as of alcohol-preferring rats (404, 455). The latter studies indicate that glia cells are targets of ethanol action and important contributors to ethanol-induced neuroplasticity.

Moreover, ethanol appears to affect different sets of genes, depending on dose, as suggested by work on animal lines selected for different ethanol-related phenotypes, i.e., preference and tolerance. These lines have been extensively studied to identify the genomic loci controlling the behavioral phenotype, an approach known as QTL analysis. Combined with genome-wide expression profiling, it can be hypothesized that if a gene product contributes to a particular phenotype through altered expression,[8] then that gene should be located within an identified QTL for this trait. The most interesting result from this combined QTL/gene expression profiling analysis is that the genetic networks controlling ethanol action at low doses, i.e., ethanol preference, are completely different from the ones involved in ethanol tolerance, which requires much higher doses of the drug (415).

Despite the fact that the brain area of interest and the applied ethanol dose are important determinants in the transcriptomic response, throughout all expression profiling studies on ethanol the dominant biological theme is related to metabolism and cellular stress response. It

[8] Differences in gene expression can arise from *cis*-regulatory changes that affect transcription initiation, transcription rate, and/or transcript stability in an allele-specific manner, or from *trans*-regulatory changes that modify the activity or expression of factors that interact with *cis*-regulatory sequences.

must be borne in mind that as well as its specific pharmacological action, alcohol is a naturally available nutrient, at least at low-to-moderate doses, and that highly conserved pathways have evolved for its rapid metabolism and the clearance of resulting reactive oxidative products. However, recent human imaging experiments have found that even at low doses ethanol (0.5 g/kg) causes a dramatic decrease (up to 30%) in brain glucose utilization (514). As blood glucose is the primary source of energy for the brain, uncompensated reduction of this magnitude would render a subject near unconscious. Thus the data imply that low amounts of ethanol cause a relative energy deficit that is substituted for by a rapid metabolic shift towards suboptimal substrates, possibly ethanol-derived acetate and, consequently, increased oxidative stress. It seems plausible that a tuning mechanism exists to ensure sufficient ATP production defense from reactive oxygen species, and that these are reflected in the large variety of differential expressed metabolic genes in microarray studies. Indeed, metabolic flexibility may present one driving force in the selection for ethanol preference and the generation of respective selected animal models (42, 454). Such a view is consistent with observations in fruit flies demonstrating that two complementary molecular pathways are necessary to confer ethanol-induced responses: the octopamine-induced pathway (a functional analog of mammalian DA) and a cellular stress pathway regulated by a transcription factor termed *hangover* (429).

In summary, in the last 5 years, a large number of new alcohol-responsive genes have been identified by microarray analysis, and it is not surprising that many genetically altered animal models have been subsequently generated to study the functional consequences of these gene alterations. A recent comprehensive review of the literature found relevant data for ~90 genes (102) and, in fact, more than half of the genetically engineered mutants demonstrated significant effects on alcohol self-administration and reinforcement measured by other methods such as conditioned place preference (499). However, it is something of a puzzle why well-characterized alcohol-responsive genes (e.g., genes that encode neurotransmitter components) frequently do not arise in microarray analyses. One shortcoming of this kind of analysis is that transcript abundance for neurotransmission-related transcripts tends to be low compared with other gene categories. Furthermore, only a low sensitivity is achieved in microarray studies. Thus minor changes in gene expression in the range of 20–30% usually cannot be reliably detected. However, a large number of genes affected by alcohol might fall in this range. To circumvent this shortcoming in microarray experiments and other limitations, such as restraint in resources, spatial resolution and issues concerning data interpretation, a recent study successfully used massive in situ hybridization to examine a large panel of functionally related genes for differential gene expression across a number of forebrain regions of alcohol-preferring msP and normal Wistar rats as well as their responses to ethanol (172). This hypothesis-driven study and its follow-up experiments demonstrated that genes related to the CRH, nociceptin, and endocannabinoid systems are differentially expressed within the extended amygdala circuit in alcohol-preferring msP rats (86, 126, 170) and that these genes are regulated by voluntary ethanol consumption (171).

Such types of data may eventually be suitable for a more systems-oriented data analysis (Fig. 8). However, modern systems biological modeling tools require sufficient numbers of data points from time and dose responses within the neuroanatomical context of the functional circuits that underlie a behavioral output. To meet such experimental demands, priority needs to be given to further integration of transcriptional analysis with in vivo electrophysiology, imaging, and other functional readouts as described in sections V and VI.

For future studies, there is great hope of identifying persistent changes in gene expression following alcohol exposure. Persistent alcohol-induced alterations in gene expression have been proposed as a "molecular switch" that could mediate lasting adaptations and maladaptations in the brain and as a consequence pathological behavior. Yet this "molecular switch," which defines the irreversible transition from controlled to compulsive drug use, has so far not been identified (457). Alternatively, it has been proposed that epigenetic mechanisms, which exert lasting control over gene expression without altering the genetic code, could mediate persistent molecular alterations within the reinforcement system (497).

B. Epigenetic Effects Induced by Ethanol

The term *epigenetics* describes heritable genetic modifications that are not attributable to changes in the primary DNA sequence. Recent developments indicate that ethanol can induce epigenetic alterations, particularly acetylation and methylation of histones, and hypo- and hypermethylation of DNA. This has opened up a new area of interest in alcohol research and provides novel insights into actions of ethanol at the nucleosomal level in relation to gene expression and pathophysiological consequences.

Homocysteine is a main component in transmethylation reactions (439) (Fig. 9). It is remethylated to methionine by methionine synthase. Methionine synthase depends on vitamin B_{12} and uses methyl-5,6,7,8-tetrahydrofolate for transmethylation. Acetaldehyde inhibits the function of methionine synthase. Acetaldehyde, the first product generated in alcohol metabolism, is produced not only in the liver but can also be produced in the brain by

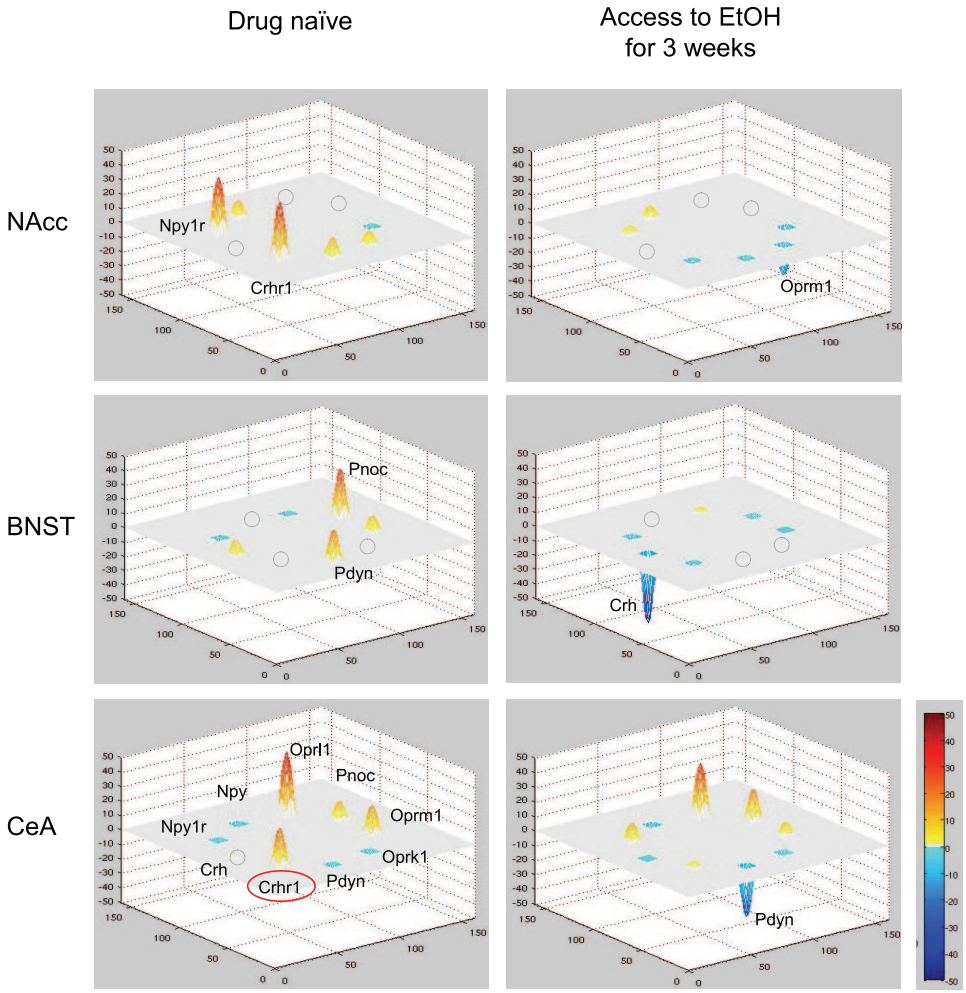


FIG. 8. Ethanol consumption changes expression landscape within the extended amygdala circuitry. Using in situ hybridization, plots show differences in gene expression of stress-related peptides and their receptors between ethanol-preferring msP rats before and after ethanol access compared with naive, normal outbred Wistar rats. Selected brain regions are related to the extended amygdala circuitry: central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), and nucleus accumbens (NAcc). The construct of the extended amygdala, which is thought to be a key target of ethanol action (248), relates to brain circuits that share some cytoarchitectural similarities (183) and are involved in mediating positive and negative reinforcement. Note that voluntary ethanol consumption in msP rats reduces the expression of many of these genes to or below normal levels. Data are normalized to naive Wistar animals using percent maximum transformation method, which allows direct comparison of each trait despite differences in basal expression levels. Color coding is from red to blue: higher or lower expression compared with naive Wistar, respectively. Open circle, no visible difference; red circle, for comparison, difference in Crhr1 expression between naive and ethanol-drinking msP is $P < 0.01$ (171). Genes: Crh, corticosterone-releasing hormone; Crhr1, corticosterone-releasing hormone receptor type 1; Pdyn, prodynorphin; Oprk1, opioid receptor; kappa 1, Oprm1, opioid receptor; mu 1, Pnoc, pronociceptin; Oprl1, (nociceptin) opioid receptor-like 1; Npy, neuropeptide Y; Npy1r, neuropeptide Y receptor Y1. (Figure courtesy of R. Momenan, A. C. Hansson, W. H. Sommer, and M. Heilig.)

the enzyme catalase after alcohol exposure (13). Acetaldehyde-mediated inhibition of methionine synthase might be one pathological mechanism leading to enhanced homocysteine levels following chronic alcohol intake, a condition called hyperhomocysteinemia. Methionine is activated to *S*-adenosyl-methionine (SAM) by ATP. SAM is one of the most potent methyl group donors in human metabolism. It is able to transfer methyl groups to cytosine residues in the dinucleotide sequence "CpG" of genomic DNA. CpG islands are genomic regions that contain a high frequency of CG dinucleotides. The "p" in CpG notation refers to the phosphodiester bond between the cytidine and the guanosine. In mammalian genomes, CpG islands are typically 300–3,000 base pairs in length. They are in and near ~40% of promoters of mammalian genes (~70% in human promoters) (426). CpG sequences are spread throughout the genome and are usually heavily methylated, whereas those occurring in CpG islands in the promoter regions of genes are less methylated. In the majority of cases, inactive genes are more heavily methylated than active ones (128), the reason being that methyl groups reduce DNA-binding capacity of transcription factors.

Elevated homocysteine levels are prevalent in alcohol-dependent patients, both in actively drinking alcoholics or in early abstinent patients (46, 47, 207). Moreover, a correlation between plasma homocysteine levels and BALs in nonabstinent alcoholics has been found. These elevated homocysteine levels decrease steadily during alcohol withdrawal. Various studies have reported a link between plasma homocysteine concentrations and DNA methylation regardless of whether gene specific or genome wide (540). Elevated genomic DNA methylation is found in patients suffering from chronic alcohol consumption compared with healthy controls (57), indicating that a state of hyperhomocysteinemia is associated with altered global gene expression.

Changes in gene-specific DNA promoter methylation caused by ethanol have also been characterized. In particular, alterations in DNA methylation in the promoter regions of α-synuclein might be an important example of maladaptive molecular responses to chronic alcohol ex-

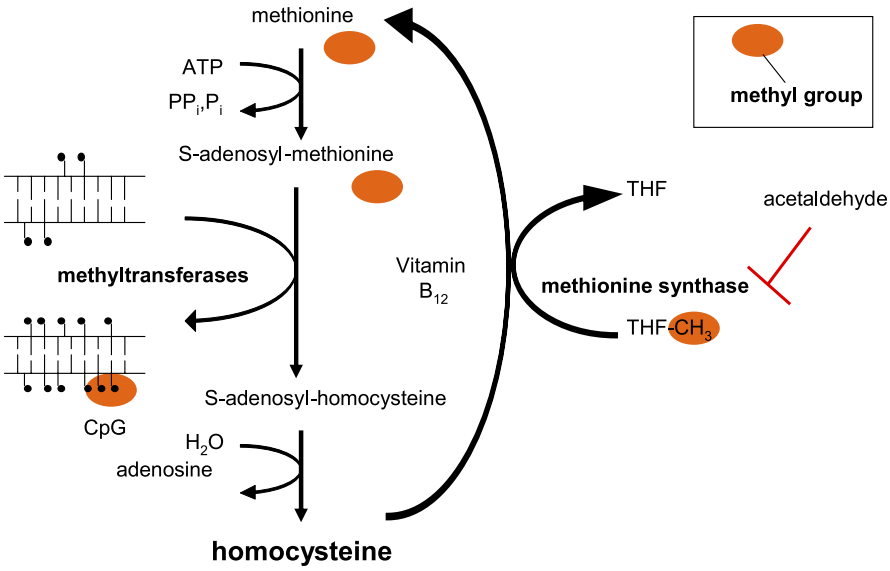


FIG. 9. Homocysteine is a major component in transmethylation reactions. It is remethylated to methionine by methionine synthase. Methionine synthase uses methyl-5,6,7,8-tetrahydrofolate (THF) + vitamin B_{12} for transmethylation. Acetaldehyde inhibits the function of methionine synthase. Methionine is activated to *S*-adenosylmethionine (SAM) by ATP. SAM is able to transfer methyl groups to cytosine residues in the dinucleotide sequence "CpG" of genomic DNA. (Figure kindly provided by S. Bleich and B. Lenz.)

posure. α-Synuclein belongs to a quantitative trait locus for increased alcohol consumption. Its expression is elevated in different brain areas of rats with inbred alcohol preference (271). It is further involved in the regulation of DA biosynthesis and DAergic neurotransmission (364). Therefore, alterations in the α-synuclein gene may have profound effects on DA-dependent alcohol seeking. In fact, increased expression of α-synuclein in alcohol-dependent patients has been observed to be correlated with obsessive craving (58). In these patients, a significant increase of the α-synuclein promoter DNA methylation was observed that was significantly associated with their elevated homocysteine levels. However, no significant differences of the promoter DNA methylation within a control gene (presenilin-1) in alcoholics and controls were found (56). These results hint at a gene-specific DNA promoter hypermethylation within the α-synuclein gene after chronic alcohol consumption. This has consequences on the protein level and, indeed, enhanced α-synuclein protein levels have been found in alcohol-dependent patients, and they positively correlated with their craving scores (56).

Very recently, in rats, it was found that alcohol exposure is associated with a decrease in HDAC activity and increases in acetylation of histones (H3 and H4), whereas during withdrawal an increase in HDAC activity and decreases in acetylation of H3 and H4 were found in the amygdala. Blocking the observed increase in HDAC activity during alcohol withdrawal with the HDAC inhibitor trichostatin A rescued the deficits in H3 and H4 in the amygdala and prevented the development of alcohol withdrawal-related symptoms such as augmented anxiety (357).

In summary, alcohol-induced alterations in methylation and acetylation patterns may have an impact on long-lasting alterations in gene expression. However, it is too premature to state whether epigenetic alterations with the α-synuclein gene constitute a molecular switch for lasting maladaptations in the brain. Nevertheless, these findings exemplify that studies on epigenetic effects induced by chronic alcohol exposure may be promising in identifying molecular mechanisms underlying addictive behavior. As discussed in the next chapter for the synaptic and cellular levels, it has however been claimed by some researchers that long-lasting alterations in synaptic plasticity have been identified that may underlie addictive behavior.

V. SYNAPTIC AND CELLULAR EFFECTS MEDIATED BY ALCOHOL

A ubiquitous property of all synapses is their ability to undergo activity-dependent changes in synaptic plasticity that can be studied most effectively using electrophysiological methods in brain slices. Since these slices only remain viable for several hours, the cellular mechanisms underlying the first few hours of LTP and long-term depression (LTD) are the best understood. It has been suggested that synaptic plasticity within the mesolimbic DAergic system and associated limbic structures, including the extended amygdala, becomes manifest following alcohol exposure (234). Some key publications on drug-induced adaptations in the mesolimbic system have revealed that glutamatergic synapses on DA neurons in the VTA, in particular, undergo plastic changes following administration of drugs of abuse including ethanol (414, 501).

By increasing synaptic strength (501), facilitating LTP (274), or blocking LTD (223), drugs of abuse augment

the responsiveness of DA neurons to glutamate and, ultimately, promote enhanced DA release in brain areas such as the NAC and the prefrontal cortex (161). Drug-induced synaptic strengthening in DA neurons in the VTA is associated with changes in AMPA receptor subunit composition (32). Incorporation of the AMPA receptor subunit GluR1 promotes drug-induced synaptic strengthening, probably through the formation of highly conductive, Ca^{2+}-permeable GluR1 homomeric AMPA receptors (119), while insertion of GluR2-containing receptors reverts it (293). Synaptic recruitment of GluR1 subunits and the resultant synaptic potentiation requires the activation of NMDA receptors (119). These synaptic changes in DA neurons are thought to be related to the development of reinforcement processes (131, 234). Very recently, it has been shown that postsynaptic AMPA receptor function in VTA neurons was significantly enhanced after alcohol self-administration (476). As increased VTA AMPA receptor function can significantly regulate firing and enhance the reinforcing effects of drugs of abuse, the increased AMPA receptor activity observed in this study may facilitate the drive to consume alcohol.

Although the VTA-NAC pathway is the most extensively studied circuit with regards to reinforcement processes, it is clear that other brain regions, especially those of the extended amygdala, are also essential components (183, 248). There is evidence that synaptic plasticity in two additional regions, the bed nucleus of the stria terminalis (BNST) and the amygdala, may also be modified by ethanol. The BNST is considered to be a component of the extended amygdala and plays a role in stress- and reinforcement-related limbic circuitry. NMDAR-dependent LTP triggering in the BNST is impaired by acute ethanol ingestion, in part through the attenuation of NMDAR-mediated synaptic currents (526).

The effects of ethanol on long-term synaptic plasticity have also been studied in the dorsomedial striatum (541), a striatal subregion that plays a central role in the acquisition and selection of goal-directed actions. Ethanol has been found to impair NMDA receptor-dependent LTP in a dose-dependent manner. At the relatively low concentration of 10 mM, a concentration comparable to mildly intoxicating BALs, LTP is abolished in the dorsomedial striatum. It has further been shown that the loss of LTP in the presence of ethanol is not due to a decrease in AMPA receptor-mediated glutamatergic transmission, a finding which is in accordance with another report showing that ethanol has only a weak effect on AMPA receptor-mediated synaptic currents in striatum (80). These results suggest that ethanol can reverse the direction of synaptic plasticity in a brain area that is critically involved in goal-directed behavior. Compensatory engagement of the alternative habit system may occur as a result of this impaired goal-directed behavior. Acute ethanol exposure, even at relatively low doses, may thus promote habit formation.

In conclusion, alcohol-induced synaptic plasticity has been found in the VTA-NAC projection as well as in other brain areas of the extended amygdala. However, the generally held view that these cellular adaptations underlie alcohol reinforcement, alcohol seeking, or alcohol-induced habit formation is based on purely associative findings. Direct experimental evidence for the behavioral significance of these drug-induced synaptic changes involving glutamate receptors is still lacking. Only in vivo electrophysiology in conditional mouse models that selectively lack, for example, NMDA receptors in DAergic neurons will provide a clear answer as to whether AMPA/NMDA receptor-induced synaptic strengthening of DA neurons within the VTA serves as a cellular model for the induction of alcohol reinforcement.

VI. NEURONAL NETWORK EFFECTS INDUCED BY ALCOHOL

A. Multielectrode Recording to Reveal Neuronal Network Activity Underlying Alcohol-Related Behavior

An increasing number of laboratories now have the capability to monitor simultaneously the extracellular activity of 100+ single neurons in freely moving animals. This paradigm, known as multielectrode recording, is revolutionizing systems neuroscience by enabling the visualization of the function of entire neural circuits (341).

So far, only a few studies have used this technique in freely moving animals to correlate alcohol-related behavior with neuronal activity. Janak et al. (217) used multielectrode recording within the shell of the NAC during operant alcohol self-administration and found that different, but overlapping, populations of neurons in the NAC mediate each event occurring along the temporal dimension of a single trial performed to obtain ethanol reward. These data suggest that the NAC plays a crucial role in linking conditioned and unconditioned internal and external stimuli with motor plans to allow ethanol-seeking behavior to occur. In a recent study, multielectrode recording was used to determine the effects of ethanol on neuronal firing and network patterns of persistent activity in PFC neurons (498). The results of this study showed that ethanol inhibits persistent activity and spike firing of PFC neurons and that the degree of ethanol inhibition may be influenced by DA D1 receptor tone. Ethanol-induced alterations in the activity of deep-layer cortical neurons may, therefore, underlie the disruptive effects of alcohol on cognitive functions supported by these neurons.

These few examples demonstrate that multielectrode recording in freely moving animals may, in the future,

prove to be a significant approach in understanding alterations of neural network activity during the course of long-term alcohol consumption. Application of this technique to investigate the transition from alcohol-seeking behavior to more compulsive behavior would be of particular value (463, 535) (see sect. VIIA). However, such studies would need to be performed over a long time period, with repeated measurements being taken over several weeks or even months; data handling and analysis would be further limiting factors.

B. Human Brain Imaging to Identify the Neuroanatomical and Neurochemical Substrates of Addictive Behavior

Major advances in alcohol research have been made as a result of progress in human neuroimaging, particularly when used in combination with psychopharmacology and molecular genetics (315, 513). Structural magnetic resonance imaging (MRI), functional imaging (fMRI), spectroscopy, and PET have elucidated mechanisms of brain damage in alcohol-dependent patients. They have also deepened understanding of neuronal networks and the contribution made by various neurotransmitter systems involved in alcohol reinforcement and addictive behavior, such as the DAergic, glutamatergic, and opioidergic systems. The combining of imaging genetics (315) and imaging pharmacology (pharmacological MRI; phMRI) (474) promises to open up new avenues of research in the study of gene × environment interactions in specific neuronal networks (457, 513).

In the search for the neuroanatomical substrates of addictive behavior, imaging techniques have provided for the first time a window into the brain of alcohol-dependent patients. Structural MRI, for example, has demonstrated macrostructural changes in the alcohol-dependent brain that are very likely to be of clinical relevance. Pfefferbaum et al. (370) have clearly documented the loss of frontocortical grey matter that occurs in alcohol-dependent individuals over time (410). Given the well-established role of the frontal lobes in decision-making and impulse control, it is clear that impairments in this region are likely to contribute to the vicious cycle of uncontrolled alcohol use. However, it remains unclear whether alcohol consumption in nondependent social drinkers affects the brain in a similar manner (370). Grey matter volume abnormalities following chronic alcohol consumption have also been detected in other areas of the brain, such as the hippocampus and amygdala. Reduced volumes in the hippocampus and amygdala, which are associated with increased externalizing symptoms such as attention deficit and hyperactivity, have been found, in particular, in young, alcohol-naive subjects at high risk of alcohol addiction (35, 191).

The fMRI approach is being increasingly applied in alcohol research. Cue exposure paradigms conducted in the scanner have demonstrated that specific brain regions become activated in alcohol-dependent subjects. Compared with social-drinking subjects, alcohol-dependent subjects were shown to have increased activity in the prefrontal cortex and anterior limbic regions after ingestion of a sip of alcohol while viewing alcohol cues. In addition, brain activity in the left NAC, anterior cingulate, and left orbitofrontal cortex has been shown to be significantly correlated with subjective craving ratings in alcohol-dependent subjects, but not in control subjects (153, 334). Cue-induced activation of these brain areas appears to be most pronounced in subjects who subsequently relapse during a 3-mo follow-up period (169), suggesting that fMRI may help to identify a group of alcohol-dependent subjects with an otherwise undetected high risk of relapse. It is of note that adolescents with alcohol abuse disorders showed substantially greater brain activation in the prefrontal cortex and anterior limbic regions in response to images of alcoholic beverages than was the case with control adolescents. Furthermore, the degree of brain response to these images was highest in those adolescents with the highest monthly alcohol consumption and who reported a greater desire to drink (483). In conclusion, a link exists between the urge to drink alcohol and fMRI responses in areas of the brain involved in mediating alcohol reinforcement, desire, and episodic recall. Use of visual alcohol stimuli demonstrates that a similar link evolves in adolescents with relatively brief drinking histories, suggesting a neural basis for the observed response to alcohol advertisements in adolescents with drinking problems.

Alcohol cues may also modulate brain responses to emotional states. fMRI was used to examine brain activation during the induction of either positive or negative mood states in conjunction with an alcohol or non-alcohol-containing beverage. In the absence of alcohol, alcohol-dependent subjects displayed more activation in response to the induction of negative as opposed to positive mood states, and greater activation than controls to negative induction (159). In the presence of alcohol, the difference in the activation of cortical networks between negative and positive mood state induction was decreased in alcohol-dependent subjects (159). This is the first demonstration of diminished brain response to negative mood state induction in the presence of alcohol cues and supports the notion that some individuals take alcohol to reduce the intensity of their negative moods (492).

The combination of fMRI and genetic analysis is expected to prove a powerful approach to the characterization of endophenotypes. Compared with genetic association studies, imaging genetics offers a more straightforward approach to associating a specific genotype with a phenotype related to alcoholism. The reason for this is

obvious: for instance, in alcohol-dependent subjects measuring an fMRI response induced by alcohol-related cues with respect to a specific genotype does not involve the interaction of many system levels (see Fig. 2 from genes to molecules to synapses to the neuronal network level). However, studying the association of a specific genotype with an artificial but certainly pragmatic diagnosis of alcoholism involves a complex level of behavior and its environmental interactions. Whereas the latter approach requires the examination of thousands of subjects in relationship to a specific gene variant to be meaningful (127), neuroimaging genetics can yield very meaningful results from the investigation of considerably less individuals. This has been elegantly demonstrated by a landmark study of amygdala activation in subjects with various genetic variants of the serotonin transporter (174). Surprisingly, this approach has yet to be applied in the field of addiction and alcohol, although the first application of imaging genetics to impulsivity and its impact on addictive behavior has recently been published (54). The tendency to choose lesser immediate rewards over greater long-term rewards characterizes addictive behavior (532). Use of a temporal discounting procedure in abstinent alcohol-dependent subjects and controls showed that immediate reward bias correlates directly with the fMRI response at several brain sites, including the dorsal PFC. In this study, the Val158Met polymorphism of the catechol-*O*-methyltransferase (COMT) gene predicted both impulsive choice behavior and activity levels in the dorsal PFC during decision-making. Although this genotype effect remained significant after controlling for a history of alcohol abuse, it demonstrates the behavioral and neuronal consequences of a genetic variation in DA metabolism. In the near future, the IMAGEN study will provide information concerning genotype/phenotype relationships in the etiology of alcoholism. IMAGEN is the first longitudinal functional and structural genetic-neuroimaging study and will investigate a cohort of 2,000 adolescents. In this prospective study, specific brain functions implicated in the etiology of disorders such as alcoholism will be linked to genetic variations and behavioral characteristics relevant to disease processes (181, 434).

A further imaging technique, phMRI, offers considerable potential for the development of new treatments. In this context, it is possible to study not only brain activation patterns triggered by alcohol-related cues or alcohol itself, but also the way in which they are modulated by anticraving drugs. A striking example of this elegant approach has recently been provided by Heilig and co-workers (154) at the NIAAA. They showed that BOLD responses elicited by alcohol-related cues were reduced by a novel neurokinin 1 receptor antagonist (154), a finding that indicates the efficacy of this drug as an anti-craving medication.

Proton magnetic resonance spectroscopy (MRS) allows quantitative and noninvasive access to a number of metabolites in various brain regions in vivo. Significant neurometabolite changes detected to date in alcohol-dependent patients are reduced *N*-acetylaspartate (NAA) and reduced choline-containing compounds (Ch). These findings were most prominent in the frontal cortex and the cerebellum, and both changes were found to be partly reversible with abstinence (34, 130, 359, 438); Ende et al. (129) found a positive correlation between the frontal Ch signal and alcohol consumption in light social drinkers. Furthermore, findings of significant differences in both NAA and Ch, occurring largely in the frontal white matter area, are in accordance with the finding that white matter loss is the most prominent structural change in the brains of alcohol-dependent subjects (175).

Another promising approach involving the use of MRS is the direct measurement of neurotransmitters such as glutamate. Measurements of central glutamate have only recently begun to appear in the literature (179, 430, 544) (Fig. 10). Measurement at 3 T is not optimal, as this is largely confined to measurement of glutamate from the metabolic pool. At 7 T, however, it is more likely that glutamate that is directly involved in neuronal communication can be measured. One important application of the measurement of glutamate in the human brain is the search for responders to the antirelapse medication acamprosate (295). Recent preclinical research demonstrated a hyperglutamatergic state in the brain of alcohol-dependent animals which is completely blunted by acamprosate treatment (106, 107, 465). Spectroscopic measures of glutamate in the human brain might therefore help to identify alcohol-dependent patients exhibiting a hyperglutamatergic state. In an initial MRS study, acamprosate or placebo was given to non-alcohol-dependent volunteers (61). In the group treated with acamprosate, NAA and glutamate signals in the brain were decreased compared with those observed in the placebo group, suggesting that acamprosate does indeed interact with glutamatergic neurotransmission in the human brain.

The DA system has been extensively studied using PET imaging. Findings from preclinical studies demonstrating that midbrain DA A10 neurons play an essential role in the acquisition of primary alcohol reinforcement processes have recently been translated to humans via PET measurements. Boileau et al. (60) examined healthy volunteers in a PET scanner following alcohol ingestion using the selective and potent DA D2/D3 receptor antagonist [^{11}C]raclopride. They found a significant reduction in [^{11}C]raclopride binding potential in the NAC, indicative of increased extracellular DA. The magnitude of the change in [^{11}C]raclopride binding correlated with the psychostimulant effects of alcohol (60).

In alcohol-dependent patients, disrupted DA function with blunted DA transmission in the NAC (301) and re-

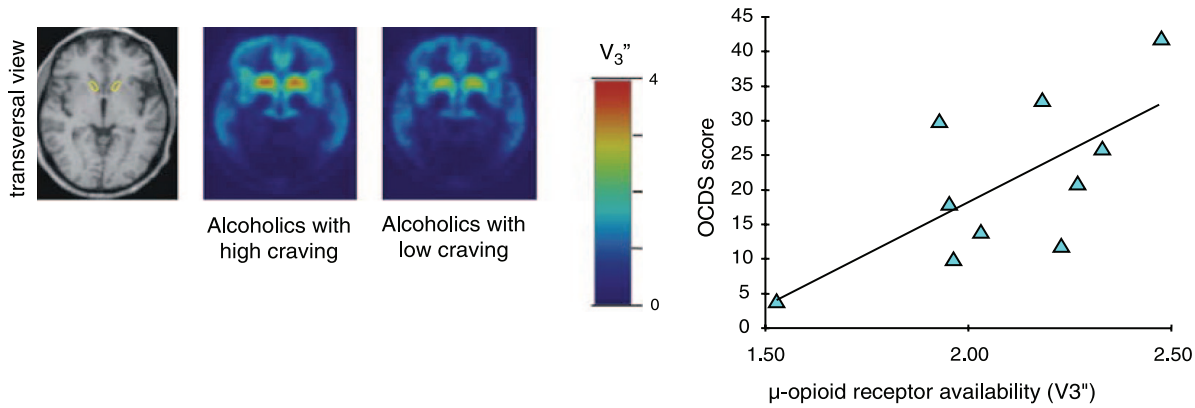


FIG. 10. Brain imaging of central μ-opioid receptor availability with a [^{11}C]carfentanil ligand. The left image defines the region of interest (NAC), and the two other images show voxel-wise averaged "V3" parametric images in alcohol-dependent patients with high and low craving, respectively. The right panel shows the correlation between μ-opiate receptor availability and severity of alcohol craving [Obsessive Compulsive Drinking Scale (OCDS) score] in alcohol-dependent patients. (Figure kindly provided by K. Mann.)

ductions in DA D2 receptor densities have been the most consistent findings, and may be related to the intensity of craving and relapse behavior (186, 513). Imaging studies in patients with type II alcoholism have revealed significant reductions in DA D2 receptor availability, and it has been suggested that low DA D2 receptor availability may represent a predisposing factor (515).[9] This is supported by the findings of a recent PET study that investigated whether high levels of DA D2 receptors may be protective against alcoholism. For this purpose, nonalcoholic subjects who had an alcoholic father and at least two other first- or second-degree relatives who were alcoholics (family-positive group) and nonalcoholic controls with no family history of alcoholism (family-negative group) were studied. A combined [^{11}C]raclopride PET to assess DA D2 receptor and [^{18}F]fludeoxyglucose PET to assess brain glucose metabolism (marker of brain function) was used. Availability of DA D2 receptors was significantly higher in the caudate and ventral striatum of family-positive subjects compared with family-negative subjects. In family-positive subjects, striatal DA D2 receptors were associated with metabolism in orbitofrontal and prefrontal cortices and personality scores of positive emotionality, but this was not the case in family-negative subjects (514). This higher than normal DA D2 receptor availability in non-alcohol-dependent members of alcohol-dependent families supports the hypothesis that high levels of DA D2 receptors may protect against alcoholism. The significant associations between DA D2 receptors and metabolism in those frontal regions involved in emotional reactivity and executive control further suggest that high levels of DA D2 receptors may protect against alcoholism by regulating circuits involved in the inhibition of behavioral response and the control of emotion (514).

A possible link between the endogenous opioid system and alcohol craving has also been studied using PET. The severity of craving following detoxification may be dependent on endorphin release and the availability of opioid receptors in the NAC. To test this hypothesis, Heinz et al. (186) recruited abstinent male alcohol-dependent subjects and age-matched healthy male controls and assessed the availability of μ-opioid receptors using PET and ^{11}C-labeled carfentanil, a radioligand that binds specifically and reversibly to μ-opioid receptors. Alcohol craving was assessed on the day of the PET with the Obsessive-Compulsive Drinking Scale (OCDS). Abstinent alcohol-dependent patients displayed an increase in μ-opioid receptors in the NAC, which correlated with the severity of alcohol craving (Fig. 10). These findings indicate the existence of a neuronal correlate with the urge to drink alcohol.

In summary, over the past decade, neuroimaging research in humans has contributed greatly to our knowledge of the neuroanatomical and neurochemical substrates of addictive behavior. In the "addicted brain," this research indicates the involvement of the extended amygdala, including the NAC, the orbitofrontal cortex, and the dorsal striatum, brain areas responsible for reinforcement, decision-making, and impulse control. Hypofunction of the DAergic system and alterations within endogenous opioid systems seem to correlate with craving and relapse behavior. Similar neuroanatomical and neurochemical findings have been observed in animal research (189, 313). Findings from preclinical studies also suggest involvement of the glutamatergic system in alcoholism (148, 496). Recent advances in glutamate spectroscopy and the development of NMDA receptor (39) and metabotropic glutamate receptor PET ligands (446) will assist in

[9] Type II people tend to become alcohol dependent at an early age and have a high family risk of alcoholism, more severe symptoms, and a negative perspective of life (59).

the translation of this knowledge to alcohol-dependent patients. The application of ultra high-field imaging in rodent models of alcoholism will provide an additional translational component in the near future.

C. Animal Brain Imaging to Identify the Neuroanatomical and Neurochemical Substrates of Addictive Behavior

Brain imaging in small laboratory animals such as mice and rats is restricted, since the brain sites of interest are very small compared with those of the human brain and measurements can only be performed in anesthetized animals. Use of a comfortable head restraint device in well-trained conscious monkeys, however, enables the performance of imaging and the assessment of conditioned drug responses (204). Nevertheless, recent progress in ultra high-field imaging up to 17 T now allows brain imaging in rodents with good resolution (<100 μm) (Fig. 11). Spectroscopy and phMRI provide particularly powerful tools for the study of the progression of alcohol consumption towards addictive behavior (see sect. VIII). The advantage of animal neuroimaging is that a subject can be studied repeatedly over a long period, allowing the investigation of neuronal network activity in the transition phase from controlled to compulsive behavior.

Glutamate spectroscopy can also be performed in laboratory animals. Pfeuffer et al. (372) demonstrated as long ago as 1999 that at least 18 metabolites, including glutamate and GABA, can be quantified in the adult rat brain using highly spectrally and spatially resolved [^{1}H]NMR spectroscopy at 9.4 T. In vivo detection and quantification of glutamate in the rat brain, as well as regional differences in signal intensities, have also been demonstrated by others (304). High-field spectroscopy provides superior peak separation (Fig. 11), allowing the direct measurement of glutamate in different brain areas of small laboratory animals, providing an ideal tool for noninvasive longitudinal tracking of neurometabolic plasticity within the glutamatergic systems accompanying alcohol withdrawal, abstinence, and relapse.

The most promising approach, however, is the in vivo mapping of functional connectivity in neurotransmitter systems using phMRI. Schwarz and colleagues (435, 436) have pioneered the application of functional connectivity studies to pharmacological challenges. In their studies, analysis of the phMRI responses to various drugs revealed specific structures for functionally connected brain regions that closely reflect known pathways in the neurotransmitter systems targeted by these drugs (435, 436). These studies therefore demonstrate that the hemody-

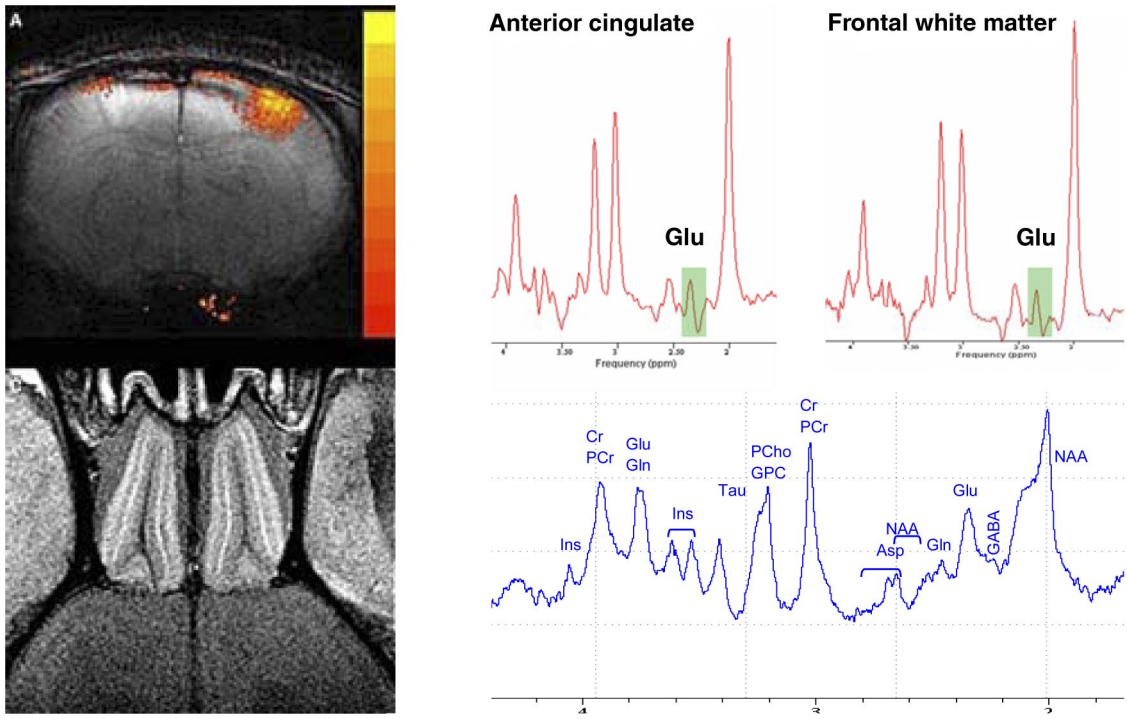


FIG. 11. High-field imaging with 11.7 T now allows brain imaging in the rodent brain. *Top left*: a BOLD-fMRI correlation coefficient map of forepaw stimulation is shown in the rat brain. *Bottom left*: T1-weighted MRI is possible at this field strength after Mn^{2+} administration. The cortical layers in the olfactory bulb and somatosensory cortex at a resolution of 100 μm are shown here. (Both figures on *left* provided by BRUKER.) *Top right*: glutamate spectroscopy in the human brain at 3 T. *Bottom right*: a striatal spectrum with a good peak separation for glutamate in the rat brain at 9.4 T. (Both spectrums were kindly provided by G. Ende.)

namic responses observed following a pharmacological challenge are closely related to drug-specific changes in neurotransmission. This novel approach can now be used to study the impact of pharmacological or genetic manipulation on functional connectivity. This application has already been used to study the disruption of drug-induced functional connectivity by a DA D3 antagonist. The strongest modifications of functional connectivity by DA D3 blockade occurred in nigrostriatal connections (437). This approach is also being applied to current alcohol research. The progression of alcohol drinking towards a habitlike behavior, as studied in terms of alteration in nigrostriatal connectivity of brain sites, is being studied in a long-term alcohol self-administration paradigm (see sect. VIII) using a 9.4-T scanner. The working hypothesis is that the nigrostriatal pathway may be involved in the habit-forming properties of alcohol and other drugs of abuse (116, 138, 156, 457). More precisely, a neuroanatomical principle of striatal organization is that ventral domains, including the NAC, exert control over dorsal striatal processes that are mediated by so-called "spiraling," striatonigrostriatal circuitry. Chronic administration of drugs of abuse may lead to alterations in this serial connectivity and, as a result, drug-seeking habits (a key characteristic of addictive behavior) are triggered (30). DA D3 receptors may play a key role in this process. A selective upregulation of DA D3 receptors in the striatum has been observed in several rat lines undergoing long-term alcohol self-administration with repeated deprivation phases (509). Administration of DA D3 antagonists in these rats decreased alcohol-seeking reponses and relapse-like drinking behavior in a dose-dependent manner (509). In conclusion, upregulation of D3Rs following long-term home-cage alcohol exposure may not be related to the alcohol intake per se, but rather to the stimulus-response habit. Functional connectivity studies with good resolution conducted in a high-field scanner provide a tool to prove this attractive hypothesis of alcohol/drug-induced alterations of striato-midbrain-striatal serial connectivity.

VII. BEHAVIORAL EFFECTS INDUCED BY ALCOHOL: FROM CONTROLLED DRINKING TO ALCOHOLISM

Alcohol drinking occurring over a long time period can be separated into three phases. The first phase is the acquisition of alcohol drinking, followed by a second phase of controlled alcohol-drinking behavior, and then follows a third phase where uncontrolled alcohol-drinking behavior occurs (463, 507). Epidemiological data from a 10-yr large-scale prospective study of a representative population sample (>3,000 subjects) revealed an alcohol-specific symptom progression model for alcoholism. This model describes transition probabilities from one phase to another (non-use, use, heavy use, abuse, addiction) in relation to biological, psychological, and social vulnerability and risk factors (365). In the past, most animal work focused on the acquisition of alcohol drinking or the maintenance of an established controlled alcohol-seeking behavior. This work, reviewed here, has led to the characterization of the neuroanatomical and neurochemical substrates of alcohol reinforcement processes. More recently, however, substantial progress has been made in modeling the third phase, in which uncontrolled compulsive alcohol consumption and seeking behavior occurs (425, 535). In this phase, positive reinforcement processes become less important. There is a shift from "liking to wanting" alcohol as habit-forming properties (138) and opponent motivational processes, mainly triggered by acute, protracted, and conditioned withdrawal, come increasingly into play (451). Subsequent allostatic dysregulation of the reinforcement system may then occur (250). One animal model that captures these different drinking phases is the long-term alcohol self-administration procedure with repeated deprivation phases (425, 463).

A. An Animal Model to Study Different Phases of Alcohol Consumption

In a long-term alcohol self-administration procedure with repeated deprivation phases, as well as food and tap water, Wistar rats receive different concentrated ethanol solutions ad libitum in four bottles per cage (5, 10, and 20% reflecting alcoholic beverages consumed by humans such as beer, wine, and spirits).[10] After 2 mo of continuous access to alcohol, the rats are deprived of alcohol for 3 days. Following this deprivation phase all alcohol solutions are presented again. This procedure is repeated monthly for the following 10 mo. The introduction of repeated deprivation (withdrawal) phases for several days/weeks is crucial in developing an addictive behavior, as the negative consequences of acute, protracted, and conditioned withdrawal triggers further drinking and induces relapse behavior (250, 451). Following a deprivation (withdrawal) phase, re-presentation of the alcohol

[10] A four-bottle paradigm has the advantage of overcoming initial preference problems. Rats usually prefer lower concentrated alcohol solutions (<6%) over higher concentrated alcohol solutions. Following a period of taste adaptation, a shift towards preference for higher concentrated alcohol solutions is observed. Furthermore, individual sensitivities and preferences to alcohol solutions are usually observed. The free choice presentation of various concentrated alcohol solutions bypasses the problem of individual preferences; in this model a rat is allowed to drink what it likes most. Indeed, in a four-bottle paradigm, high alcohol intake and preference in common stock rats are observed during the acquisition of alcohol drinking behavior in male (444) as well as in female rats (147). In conclusion, a four-bottle paradigm results in a higher daily alcohol intake and preference compared with a two-bottle choice paradigm with a fixed alcohol concentration of 10% which has been used in most of the studies on alcohol drinking behavior in the rat performed to date.

solutions leads to a pronounced transient rise in alcohol intake and preference. This is termed the alcohol deprivation effect (ADE). This relapselike drinking phenomenon is observed across several species including rats, mice, monkeys, and human social drinkers (66, 419, 448). The increase in alcohol drinking probably reflects an increase in alcohol seeking, which, according to the self-reports of some alcohol-dependent subjects, can also increase progressively during abstinence and decrease after relapse during a drinking bout. In summary, the ADE in long-term voluntary alcohol-drinking rats is used as a measure of high motivation to drink alcohol and as a measure of relapse-like behavior.

In this long-term drinking model, changes in alcohol-drinking behavior occur over time. During the first days of alcohol exposure, male rats have a high daily consumption of ~6 g/kg and an alcohol preference of 60% (444, 463).[11] After this short initiation phase, large daily fluctuations in drinking behavior are observed, although over a period of months, there is a clear tendency for a decline in alcohol consumption, resulting in a stable average daily intake of between 3 and 4 g/kg alcohol. In the first 8 wk of the acquisition phase of alcohol drinking, there is a clear sequence in preferences for the different concentrated alcohol solutions: 5% >> 10% > 20%. However, from week 9 onwards there is a change in this sequence to 5% < 10% ≤ 20%. This change in preferences coincides with the introduction of the first alcohol deprivation period, and this relation remains stable for up to 1 yr. Alcohol-drinking behavior during this time can be regarded as controlled (phase of maintenance). However, following repeated ADEs, alcohol-drinking behavior can become uncontrolled and compulsive. Uncontrolled drinking behavior can be assessed by the adulteration of the alcohol solution with quinine, thus altering its taste. In this experiment, quinine is added to the alcohol solution, but not to the water (460). Quinine is a very bitter tasting substance that usually produces a strong taste aversion in rats. However, despite the disagreeable taste, the long-term alcohol-drinking rats consume large amounts of the quinine-containing alcohol solution following a deprivation phase. In fact, alcohol intake and preference and the time course of the ADE of quinine-exposed animals are similar to those of control animals that have experienced the same experimental history and received unadulterated alcohol (460; Vengeliene, unpublished results). In long-term alcohol-drinking rats, alcohol intake following a deprivation period is thus relatively resistant to modification by taste adulteration, i.e., drinking behavior becomes compulsive and uncontrolled.

This conclusion is further supported by pronounced changes in the diurnal rhythm of drinking activity observed after alcohol deprivation in chronic-drinking rats. Rats were tested in a fully automated electronic drinkometer device (196) that monitors drinking patterns online. In the experiment, age-matched control animals exhibited normal drinking activity, i.e., high drinking activity during the active night phase and low, and, for some hours, absent drinking activity during the inactive light phase. In contrast, the pattern of drinking activity changed in the chronic-drinking rats during the ADE. In particular, most of the animals still showed high drinking activity during the inactive phase, and some animals even showed no differences in drinking activity during the dark and light phases of the daily cycle. Such a level of drinking activity is far beyond the normal controlled behavior seen in the appropriate control animals and indicates alterations in circadian rhythmicity and clock genes (366; see sect. VIII*C*).

In summary, alcohol consumption behavior following long-term consumption and subsequent deprivation is characterized by changes in the alcohol intake patterns of animals. The animals not only consume more alcohol, but also large amounts of highly concentrated alcohol solutions at inappropriate times during their daily cycle in an uncontrolled and compulsive manner, e.g., during the light phase when the animals are normally inactive and drinking activity is low. Finally, the fact that the clinically effective anti-relapse drugs acamprosate and naltrexone reduce or even abolish the ADE (468) lends predictive value to this animal model for the development of novel and improved drugs for the treatment of craving and relapse (see sect. IX).

B. An Animal Model to Study Alcohol-Seeking Behavior

To date, the most common procedure used to study alcohol-seeking behavior has been the so-called reinstatement model (442). In this procedure, an animal is trained to self-administer alcohol and is then subjected to extinc-

[11] Overall, female rats in our studies consume greater amounts of alcohol than male rats (147). This is in accordance with previous studies reporting that there is a sex difference in ethanol ingestion (7, 258) and that female rats consume significantly greater amounts of alcohol. Such a sex difference is also seen in other species such as mice and monkeys (25, 362). At first glance, this appears to be in stark contrast to observations in humans, since epidemiological and clinical studies demonstrate that women consume less alcohol than men. However, we have recently reported that if alcohol intake in humans were to be calculated on a g/kg basis instead of the number of drinks consumed, consumption in females would be much the same or even more compared with that in males (239). Contrasting sex differences in humans and animals are mainly related to social barriers in different populations and to an artifact in calculating exact alcohol intake. The reasons for sex differences in alcohol consumption are still poorly understood. However, it is obvious that intrinsic sex differences in brain organization and the actions of circulating gonadal steroids may contribute to the enhanced voluntary alcohol intake observed in female animals (7).

tion, i.e., the animal is tested under conditions of nonreinforcement until operant responding appears to be extinguished. When the animal reaches a certain criterion of unresponsiveness, various stimuli are presented. A stimulus is said to have reinstated the alcohol-seeking behavior if it causes renewed response, i.e., lever pressing, without any further response-contingent alcohol reinforcement (for an illustration of this model, see Ref. 425). Reinstatement can be induced by a small quantity of alcohol. This phenomenon is consistent with the widely reported description of the "first-drink" phenomenon by which ingestion of a small amount of alcohol may induce a strong subjective state of craving in abstinent alcohol-dependent subjects (285). This priming effect can even occur in alcohol-dependent subjects who have abstained for years (36). Stresses caused by intermittent mild electric shocks to the animals' feet (266) as well as alcohol-associated olfactory cues (232) can also reinstate previously extinguished response for alcohol. Data derived from studies using the reinstatement model suggest that the neuronal substrates mediating alcohol-, stress-, and cue-induced reinstatement are not identical (276, 442). This indicates that more than one neurobiological pathway is involved in provoking alcohol-seeking behavior. Importantly, the reinstatement model has also been validated pharmacologically. Acamprosate and naltrexone are known to reduce craving and relapse in alcohol-dependent patients and can also reduce or even block cue-induced reinstatement of alcohol-seeking behavior (17, 232). Stress-induced reinstatement can be mimicked by yohimbine administration and can be blocked by CRH1 receptor antagonists, whereas naltrexone has no impact on this behavior (297). The reinforcement model is, therefore, also frequently used for the development of novel and improved drugs for the treatment of craving (see sect. IX).

In summary, the last decade has witnessed advances in the field of alcohol research with the development of new animal models mimicking core features of an addictive behavior. The validity of animal models is typically assessed using three evaluation criteria, including face, construct, and predictive validity. Reliability is also a critical issue in complex animal models. At the present time, the reinstatement and alcohol deprivation paradigms are the models for which these issues have been addressed most systematically (457). Another animal model in which excessive drinking following a history of dependence is used by several laboratories to study the neurochemical substrates of the "addicted brain" (394, 395, 398). In this model dependence is induced by subjecting animals to a 4-wk period of intermittent vapor exposure during which they are exposed to ethanol vapor for half of the day. Following dependence induction, pharmacological or genetic manipulations can be made to modulate augmented self-administration of ethanol in postdependent rats. The increase of ethanol self-administration in this animal model is hypothesized to involve an allostatic-like adjustment in which the set point for ethanol reward is enhanced (250).

Considerable work remains to be done to establish whether measures obtained in these and other models are valid and reliable. The refinement of these animal models and the characterization of specific reliable phenotypes within these models is a challenging process that requires a multidisciplinary research approach, involving collaboration between experimental and clinical psychologists, clinicians and, of course, the patients themselves. Nevertheless, despite the negative consequences, these models can already be used to study the neurobiological foundation of the reinstatement of alcohol-seeking behavior, relapse, loss of control, and drug intake.

VIII. COMORBIDITY, GENETIC, AND ENVIRONMENTAL FACTORS THAT CONTRIBUTE TO ALCOHOL USE AND ADDICTIVE BEHAVIOR

Susceptibility factors that substantially increase the risk of developing alcohol addiction include concomitant psychiatric disorders, such as anxiety and major depressive disorders. Posttraumatic stress disorder (PTSD) is also frequently associated with alcoholism. In a recent population-based, longitudinal descriptive study of 88,235 United States soldiers returning from Iraq, PTSD was often associated with alcohol-related problems (320). There are also known personality traits, such as passive-dependent, impulsive, or antisocial traits that lead to an inidvidual's differential response to novelty, punishment, and reward and to adaptive responses to environmental challenges in general (90). In particular, antisocial personality disorder is associated with a high degree of alcoholism (431). These psychiatric disorders and personality traits are thought to reflect differences in brain neurotransmitter systems which, in turn, influence the pharmacodynamics of alcohol and determine, at least in part, an individual's liability to seek alcohol reward and to become addicted to it after long-term and excessive exposure.

A. Anxiety and Alcohol Drinking/Addictive Behavior

Apart from the reinforcing and discriminative stimulus effects of alcohol, its anxiolytic effects may also play a role in motivating its ingestion, at least in individuals who are susceptible to the anxiolytic action of alcohol (83, 462, 547). This is based on the so-called "tension reduction hypothesis" of Conger (97), which proposes that alcohol consumption may be found to be anxiety-

reducing, which then reinforces alcohol consumption and promotes future alcohol intake, i.e., the ingestion of alcohol may be an attempt to self-medicate against anxiety symptoms. In conclusion, anxiety may trigger alcohol consumption. Alternatively, alcohol intake may also cause the development of anxiety symptoms. Indeed, clinical observations show that increased quantities of alcohol consumed per drinking session are associated with increased symptoms of anxiety in the sober state (75), and withdrawal from alcohol, which can be conceptualized as a rebound phenomenon of the CNS from recent alcohol consumption, has been shown to be anxiogenic in both humans (406) and in rats (196). Furthermore, repeated alcohol withdrawal episodes potentiate subsequent withdrawal symptoms (29, 124), indicating a sensitizing effect of the repeated experience of withdrawal (64, 456). In terms of withdrawal-induced anxiety, it has also been shown that alcohol self-administering rats exhibit a more pronounced anxiogenic response after repeated withdrawal episodes than is the case after the first withdrawal experience (196). The latter study argues that those enhanced anxiogenic responses may contribute to more compulsive behavior. In humans, repeated alcohol withdrawal episodes may also augment anxiety, craving, and dysphoria, and this negative affective state can contribute to the continuation of alcohol drinking (124). In conclusion, anxiety experienced during alcohol withdrawal, which may be intensified by repeated experiences of such withdrawal, promotes drinking and relapse behavior. The observation that alcohol-dependent patients with a coexisting anxiety disorder have more frequent and more severe relapses supports this conclusion.

Because of the mutual interaction between anxiety and alcohol, it is possible that anxiety disorders promote the development of alcoholism and, vice versa, that alcoholism promotes the development of anxiety disorders. Epidemiological investigations addressing the issue of primary versus secondary onset have so far yielded inconsistent results. Recent investigations differentiating between subtypes of anxiety disorders have not demonstrated a consistent temporal pattern for alcoholism in relation to these disorders (481). Epidemiological data have indicated a temporal relationship underlying comorbidity between alcoholism and panic and phobic disorders, particularly social phobia (481, 547). Thus panic and social phobia are predictors of subsequent alcohol problems among adolescents and young adults, but they rarely occur after the onset of alcoholism. These findings are consistent with the notion that alcohol drinking may be used to self-medicate social phobia, and may therefore serve as a salient risk factor for the subsequent onset of problem-drinking behavior.

What can animal models tell us about the relationship between anxiety and alcohol intake? It has been shown that elevated measures of anxiety correlate with high voluntary alcohol consumption during the initiation of alcohol-drinking behavior in Wistar rats (462) and that central amygdala lesions reduce both experimental anxiety and voluntary alcohol intake in male Wistar rats, indicating a role for the central amygdala in the link between anxiety and alcohol drinking (323). Alcohol-preferring rat lines would, therefore, be predicted to be more anxious than their nonpreferring counterparts. Although this holds true for Sardinian alcohol-preferring (93) and Marchigian Sardinian alcohol-preferring rats (83), Indianapolis P-rats are less anxious than their nonpreferring counterparts (473). When all the comparative studies between multiple alcohol-preferring and nonpreferring lines are taken into consideration, the hypothesis that alcohol-preferring rats drink alcohol to reduce high anxiety states must be rejected; if anything, there appears to be a negative correlation (353, 511). This conclusion is supported by a further experimental approach. Recently reported is the establishment of two Wistar rat lines selectively bred for differing behavioral performances on the elevated plus-maze (259). The selective breeding resulted in animals with high-anxiety-related behavior (HAB) and low-anxiety-related behavior (LAB). Both lines were subjected to an alcohol preference test. Male animals did not differ in either the initiation of alcohol drinking or in relapse-like drinking following an alcohol deprivation phase (188). In contrast, female LAB rats initially showed a higher alcohol consumption and preference than female HAB rats and exhibited more pronounced relapse-like drinking behavior (188). These experiments show that, in rats, innate increased levels of anxiety can be negatively correlated with alcohol drinking and that sex can play a role in these behavioral patterns.

In summary, animal research and epidemiological studies demonstrate the existence of a complex relationship between anxiety, alcohol drinking, and addictive behavior. More refined animal models relevant to clinical phenotypes such as panic and social phobia are required to identify the neurochemical substrates underlying these more specific comorbidities.

B. Depression and Alcohol Drinking/Addictive Behavior

The comorbidity of alcoholism and depressive disorders has been extensively documented in both epidemiological and clinical investigations (10, 314, 481). While alcoholism is more common in men, epidemiological data clearly demonstrate that unipolar depression is approximately twice as common in women as in men (256, 525) and that comorbid alcoholism and depression is also more common in women than in men (109, 118, 190). This association may be based on common neurobiological factors mediating depression and alcoholism (300). However, depression can be effectively treated with antide-

pressants, whereas the use of these drugs is very limited in the treatment of alcoholism. No consensus has been reached regarding the specific mechanisms underlying the association of these disorders, and it remains unclear whether one of the disorders causes or predisposes to the other.

The relationship between high alcohol intake and a depressive-like state has been studied in alcohol-preferring rat lines; however, results have been inconsistent. Some studies indicate a positive correlation between high alcohol intake and a depressive-like state, whereas others do not (85, 243, 355). The relationship between inherent depressive-like behavior and alcohol drinking has been studied in male and female helplessness (cLH) and congenital nonlearned helplessness (cNLH) rats, selected on the basis of their behavior in learned helplessness testing (516). The acquisition and maintenance of alcohol-drinking behavior and the effect of alcohol deprivation was examined in both lines and genders (510). Alcohol intake by male cLH and cNLH rat lines did not differ significantly. In contrast, female cLH rats consumed higher amounts of alcohol than female cNLH rats. Following an alcohol deprivation phase, a significant transient increase in voluntary alcohol intake and preference ensued in both male and female rats, although the magnitude of the ADE was similar in both cNLH and cLH animals (510).

In summary, cLH rats display reduced sensitivity to rewards associated with learned helplessness (516). Reduced sensitivity to rewards, which is used as a measure for anhedonia, might explain why cLH animals consume more alcohol compared with the cNLH line. This relationship is sex specific, however, and only female animals consume more alcohol. Currently, it is not clear which neurobiological mechanisms in the reward pathway drive these sex differences. However, there are some similarities to the situation in humans since female alcohol-dependent patients are more likely than their male counterparts to suffer additionally from primary or secondary depression (109, 118, 190).

C. Gene × Environment Interactions and Alcohol Drinking/Addictive Behavior

Alcohol use has a strong genetic component, and numerous genes (>50 genes) have been shown to be involved in alcohol reinforcement and the acquisition of alcohol (102). A genetic component is well established with regard to vulnerability for alcohol use and subsequent abuse and addiction. Compared with the offspring of nonalcoholic parents, the offspring of alcoholics have a 4- to 10-fold increased probability of developing alcoholism (306, 432). Twin, adoption, and sibling studies have shown that genetic influences are directly responsible for some of the interindividual differences observed in the predisposition to alcoholism. A meta-analysis, which included 9,987 monozygotic and dizygotic twin pairs, estimated the heritability of alcoholism to be ~50–60%.

As with most psychiatric disorders, alcoholism is a complex disorder that shows no obvious Mendelian pattern of transmission and for which there is no evidence for major gene effects. This genetic complexity may be based on two parallel mechanisms: *1*) poly-/oligogenicity, a concept which assumes that functional variations at several genes (which may act via different neurobiological pathways) result in a simultaneous impact which then confers vulnerability; and *2*) heterogeneity, a concept which assumes that a single genetic variation may result in one specific phenotype that may be relevant to the acquisition and/or maintenance of addictive behavior (434). These two mechanisms are partly responsible for the fact that the contribution of single genes to the clinical phenotype of alcoholism is small.

Addictive behavior, however, is not merely the result of an adverse combination of risk alleles. Ultimately, it is the result of cumulative responses to alcohol exposure, an individuals's genetic and epigenetic make-up, and environmental perturbations over time. In fact, a variety of environmental factors contribute to the development of addictive behavior, most importantly prenatal alcohol exposure, prenatal stress, and severe stressful life events. Severe stressful experiences, such as the death of someone close or job loss, usually accompany a destabilization in personal circumstances and negative mood states. In such changing life situations, alcohol use, particularly heavy use, can reduce the intensity of negative mood states and, in the initial stages, dampen unpleasant physiological phenomena such as sleeplessness or restlessness (378). In some individuals, alcohol drinking is therefore an attempt to cope with stress (97, 548), although the relationship between stress and alcohol drinking observed in studies in humans (378) and laboratory animals (377) is much more complex than that. Accordingly, life stress is regarded as a major environmental risk factor for alcoholism. The biological explanation for this phenomenon is most likely to be that prenatal and postnatal stress can alter the activity of the hypothalamic-pituitary axis (HPA). As a result, long-lasting changes in glucocorticoid levels may occur that influence mesolimbic DAergic activity and reinforcement processes (375). Glucocorticoids have a facilitatory role in voluntary alcohol consumption, demonstrated by the finding that adrenalectomy causes a decrease in alcohol drinking in both Wistar rats (142) and alcohol-preferring AA rats (141), whereas intracerebroventricular infusion of glucocorticoids increases voluntary alcohol intake in animals (142). Given these findings, life stress-induced alterations in HPA activity may well account for the observation that stressful life events can trigger heavy drinking, alcohol abuse, and addictive behavior in humans (112). What is more, genetic variations

of molecular components of the HPA system may add to the gene × stress interactions involved in alcohol use and addictive behavior (89).

A recently identified example of a gene × stress interaction related to HPA activity is that involving the CRH1 receptor, which mediates endocrine and behavioral responses to stress (493). Mice lacking a functional CRHR1 were studied in a free-choice paradigm. Water and an alcohol solution that was given at increasing concentrations were offered as drinking fluids. The genotypes did not differ in their daily intake of alcohol. All mice were then repeatedly exposed to a social defeat stress and a forced swim stress. During these stress episodes, no differences in alcohol intake compared with baseline drinking were observed in either the wild type or knockouts. After a period of ~3 wk, however, the alcohol intake of the knockout mice began progressively to increase. This increased alcohol intake in the knockouts persisted and was still present 6 mo after exposure to the second set of stressors. In comparison, those knockouts with long-term voluntary access to alcohol that had not been exposed to the two sets of stressors displayed no changes in alcohol intake over time (445). In summary, knockout mice that lack a functional CRH1 receptor do not differ from wild-type mice in alcohol intake and preference under stress-free housing conditions. After repeated stress, however, the knockouts increase their alcohol consumption, which is then maintained at an elevated level throughout their life span. In a similar vein, a lowered threshold for stress-induced reinstatement of alcohol seeking in alcohol-preferring msP rats was described. These animals show a genetic variation of the *Crhr1* promoter that is accompanied by increased CRH1 receptor density (172). This shows that *Crhr1* genotype and expression interact with environmental stress to reinstate alcohol-seeking behavior. In conclusion, this is one of the first striking gene × environment interactions to have been demonstrated for alcohol consumption and reinstatement behavior. From these findings it can be assumed that alterations in the human CRH1 receptor gene (*hCRHR1*) might constitute a genetic risk factor for alcoholism, particularly when associated with stressful life events; indeed, human genetic studies have been able to establish such a link. Following determination of allelic frequencies of 14 polymorphisms of the *hCRHR1* gene, two haplotype tagging single nucleotide polymorphisms (htSNPs) which discriminate well between haplotypes were identified. Two independent samples were then genotyped and systematically examined for association with the htSNPs of *hCRHR1* and an association of these genetic variations of the *hCRHR1* gene with specific patterns of alcohol consumption was found (495). In a second study, data were collected as part of the Mannheim Study of Children at Risk, an ongoing epidemiological cohort study of the outcome of early risk factors from infancy into adulthood (48). In this cohort, drinking behavior and stressful life events were assessed. The adverse life event items addressed all areas of young adult life, i.e., transition from school to job, partner, family, parents, living conditions, legal problems, and health problems. In addition, an assessment of all negative life events occurring over the previous 3 yr was obtained by means of a standardized interview with the parents. Interactions between the two htSNPs covering the *hCRHR1* gene and adverse life events with respect to heavy drinking in adolescence were then studied and a gene × environment interaction was detected (48). These findings provide the first evidence in humans that the *hCRHR1* gene interacts with exposure to stressful life events and may predict heavy alcohol use in adolescents.

CRH regulates endocrine responses to stress via the HPA, and also mediates stress-related behavioral responses via extrahypothalamic sites, particularly the amygdala. To dissect out the role of the HPA and extrahypothalamic sites in enhanced and delayed stress-induced alcohol drinking, forebrain-specific *Crhr1* knockout mice were studied. In the conditional mutants, no enhanced and delayed stress-induced drinking occurred, suggesting that CRH1 receptors within the HPA are responsible for this phenomenon (A. Molander, unpublished results). CRH1 receptors within the amygdala seem to have an opposing role since their pharmacological blockade can reduce stress-induced alcohol consumption (181, 249). Another important regulator of stress-related behavior is the NPY, and CRH and NPY exert a reciprocal regulation of responsiveness to stressful stimuli. An interaction between NPY and CRF within the amygdala may be critical in maintaining a normal homeostatic emotional state (182). It has recently been shown that haplotype-driven NPY expression predicts brain responses to emotional and stress challenges. NPY haplotypes predicted levels of NPY mRNA in postmortem brain and lymphoblasts. Lower haplotype-driven NPY expression predicted higher stress-induced activation of the amygdala. A functional SNP located in the promoter region alters NPY expression in vitro and seems to account for more than half of the variation in expression in vivo (546). In addition to this striking finding, it has been repeatedly shown that NPY plays a crucial role in the control of alcohol consumption. Thiele et al. (490) reported that NPY-deficient mice show increased voluntary alcohol consumption compared with wild-type mice. In contrast, transgenic mice that overexpress NPY in neurons have a lower preference for ethanol (490). These data provide direct evidence that alcohol consumption and resistance are inversely related to NPY levels in the brain. Studies now need to be conducted to study this specific functional SNP in the NPY gene promotor in association with adverse life events and alcohol consumption.

Another interesting stress × gene interaction is related to the internal clock. A variety of physiological and behavioral processes, including alcohol consumption, display circadian rhythmicity and are driven by the expression of the circadian clock genes (464). Recent studies in knockout mouse models have revealed that the activity of these genes influences alcohol reinforcement and consumption, findings which are also supported by human genetic studies (366, 465). Conversely, alcohol and stressors have the potential to influence the expression of clock genes. It has been shown that chronic ethanol administration induces persistent upregulation of the expression of the *Period 2* (*Per2)* gene in the rat frontal cortex and striatum (78). Adult rats also display altered circadian expression of *Per* genes in β-endorphin-containing neurons in the arcuate nucleus following prenatal alcohol exposure (79). Thus prenatal alcohol exposure may have life-long consequences on the clock machinery that governs the circadian function of β-endorphin neurons and may thereby influence reinforcement processes during adolescence and adulthood. Finally, it has recently been demonstrated that severe stressors may increase *mPer1* gene expression in mice (538), a crucially important finding in the context of environmental stressors. Although clock genes of the *Per* family have been implicated in regulating alcohol-drinking behavior, these genes have, to date, only been known to mediate gene × environment interactions by physiological integration of light-darkness cycles (6, 391). However, novel evidence for the involvement of *Per1* in a stress-mediated gene × environment interaction has recently been found. An association has been discovered between a functional polymorphism in the promoter of the *hPer1* gene and increased alcohol drinking in adolescents suffering from severe adverse life events in early childhood (434). These results were validated in a *Per1* knockout model in which various stressors such as social defeat stress and forced swim stress were applied during voluntary alcohol home-cage drinking. Following these stressors, augmented stress-related drinking was observed in *Per1* knockout mice as opposed to their wild-type littermates. This phenomenon seems to be associated with altered expression of prodynorphin in the amygdala (Spanagel et al., unpublished results). These data identify a novel function of the circadian rhythm gene *Per1* by describing a gene × stress interaction.

In conclusion, more examples from preclinical and human genetic studies demonstrate a stress-related gene × environment interaction (89). These genes are related to endocrine HPA activity (240) and emotion regulation by the amygdala (25, 182). Furthermore, these studies demonstrate that it is only gene × environment interactions that ultimately drive the behavioral and pathophysiological responses to chronic alcohol exposure, as outlined in Figure 2.

IX. TREATMENT ASPECTS

The aforementioned complex gene × environment interactions not only lead to a large clinical heterogeneity in terms of symptom dimensions and severity of alcoholism but also to large variability in treatment response. In fact, only 20–30% of treated patients respond to so-called anti-craving and anti-relapse compounds. Therefore, in the future, an individualized approach is warranted, which calls for a real need for surrogate clinical readouts; either molecular (biomarkers such as genetic markers, peripheral protein markers, and metabolites) or endophenotypes, which could be used to predict treatment response for those medications.

A. Preclinical Medication Developments for the Treatment of Craving and Relapse

As pharmacological treatment strategies for craving and relapse behavior have recently been extensively reviewed (180, 295, 461, 468), an overview of recent preclinical findings is not presented here. Figures 12–17, however, summarize all results published for the ADE and reinstatement models. As outlined in section VII, these models can be used to study the neurobiological basis of the reinstatement of alcohol-seeking behavior and relapse-like behavior. The fact that these animal models have been positively validated using the clinically effective medications acamprosate and naltrexone is of crucial importance, since this means that their predictive validity is high. Positive testing of new putative compounds in both of these animal models provides a good rationale for further translational studies and randomized controlled trials (RCTs). Numerous compounds have produced positive signals in the ADE and reinstatement testing; however, there has been one striking exception. Administration of a κ-opioid receptor agonist has been observed to produce a potentiation of the ADE (198). Since κ-opioid receptor activation has pronounced aversive motivational consequences in animals (22, 330) and produces marked "dysphoria" in humans (371), it has been suggested that increased alcohol consumption following administration of a κ-opioid receptor agonist may be an attempt to counteract the aversive effects of this treatment. These studies highlight the importance of anti-reward pathways and further demonstrate the importance of alterations of prodynorphin and κ-opioid receptor signaling in producing negative mood states. Recruitment of these anti-reward mechanisms seems to have a pronounced impact during both protracted and conditioned withdrawal. In an animal model demonstrating excessive alcohol consumption induced by such a postdependent state (250, 398), pharmacological blockade of κ-opioid receptors led to a significant reduction in high alcohol intake (518). This

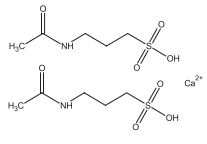
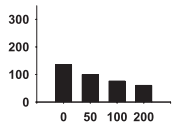
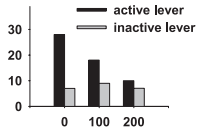
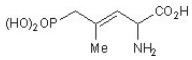
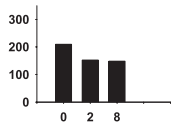
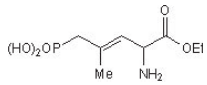
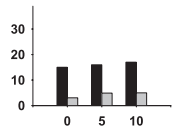
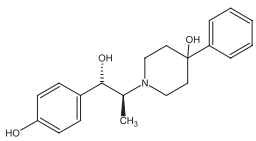
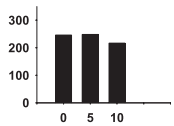
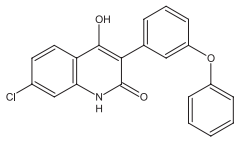
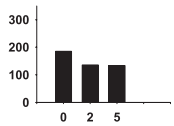
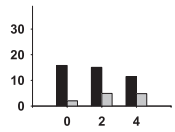
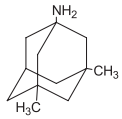
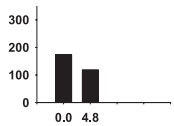
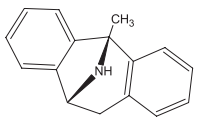
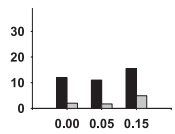
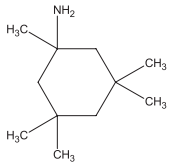
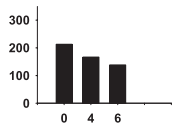
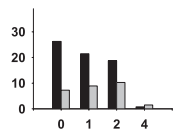
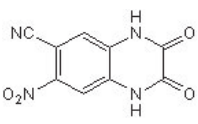
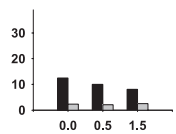
Name	Chemical Structure	ADE	Reinstatement
Acamprosate functional NMDA receptor antagonist			
CGP37849 competitive NMDA receptor antagonist			
CGP39551 competitive NMDA receptor antagonist			
Ifenprodil NMDA NR2B antagonist			
L-701.324 NMDA GlycineB antagonist			
Memantine non-competitive NMDA receptor antagonist			
MK-801 (dizocilpine) non-competitive NMDA receptor antagonist			
Neramexane non-competitive NMDA receptor antagonist			
CNQX competitive AMPA/KA receptor antagonist			

FIG. 12. The efficacy of putative anticraving and antirelapse compounds. Various classes of compounds are shown with regards to their effects in the alcohol deprivation effects (ADE) and reinstatement model. For ADE measurements, the *y*-axis represents ethanol intake as a percentage difference from baseline drinking, which is set at 100%. For this purpose, the data of home-cage drinking or operant ethanol self-administration were used. For cue (or stress)-induced reinstatement of ethanol seeking, the number of active lever responses are shown. For control conditions, inactive lever responses are also shown whenever the data were given in the original publication. For ADE and reinstatement measures, the *x*-axis represents the dose of the compound (mg/kg) administered (unless stated otherwise). *A*: agents acting at glutamate receptors. *B*: top 5 are agents acting at glutamate receptors, and bottom 4 are agents acting at GABA receptors. The following references were used for the different classes of compounds: agents acting on glutamate receptors, Refs. 17–20, 195, 405, 423, 464, 506; agents acting on GABA receptors, Refs. 70, 95, 427. (Figure produced by Valentina Vengeliene.)

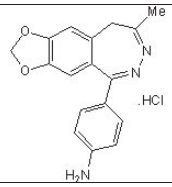
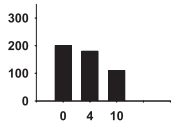
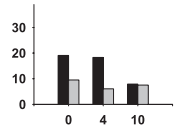
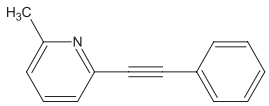
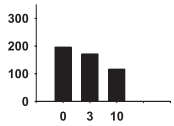
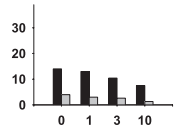
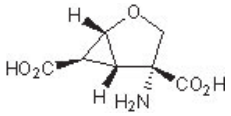
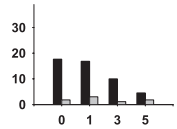
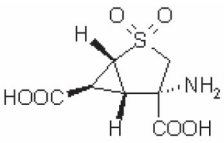
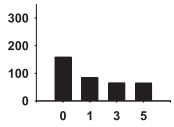
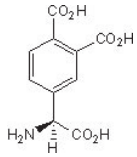
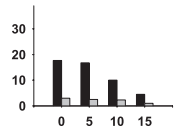
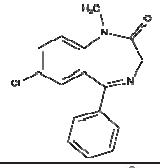
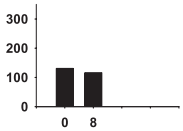
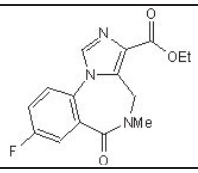
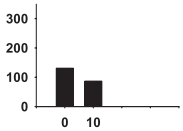
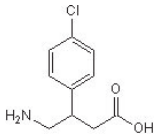
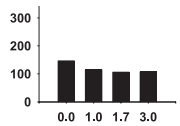
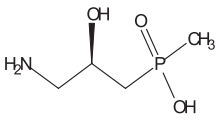
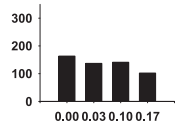
Name	Chemical Structure	ADE	Reinstatement
GYKI 52466 non-competitive AMPA/KA receptor antagonist			
MPEP uncompetitive mGluR5 receptor antagonist			
LY 379268 group II mGlu receptor agonist			
LY404039 group II mGlu receptor agonist			
(S)-3,4-DCPG mGluR8 agonist			
Diazepam $GABA_A$ receptor antagonist			
Flumazenil $GABA_A$ receptor antagonist			
Baclofen $GABA_B$ receptor agonist			
CGP 44532 $GABA_B$ receptor agonists			

FIG. 12.—*Continued*

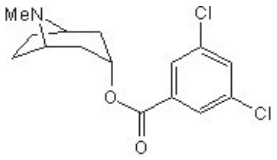
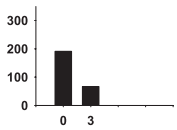
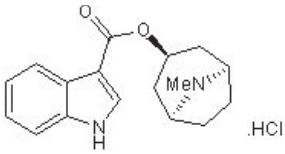
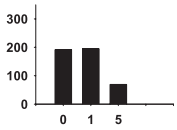
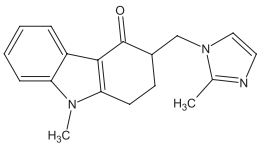
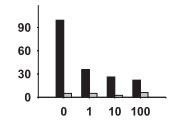
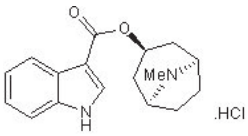
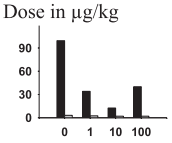
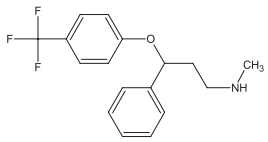
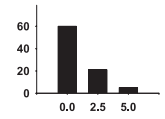
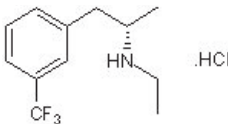
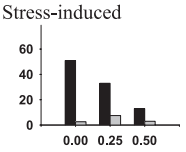
Name	Chemical Structure	ADE	Reinstatement
MDL 72222 5-HT_3 receptor antagonist			
ICS 205-930 5-HT_3 receptor antagonist			
Ondansetron 5-HT_3 receptor antagonist			
Tropisetron 5-HT_3 receptor antagonist			
Fluoxetine selective 5-HT reuptake inhibitor			
Dexfenfluramine 5-HT releaser and reuptake blocker			

FIG. 13. Agents acting on serotonin receptors (details are as in Fig. 12); see Refs. 264, 265, 403. (Figure produced by Valentina Vengeliene.)

suggests that κ-opioid receptors may also play a role in alcohol relapse and craving, although administration of the κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) did not reduce the ADE (Figs. 12–17) (198). Further studies are required, especially with regard to administration of κ-opioid receptor ligands during reinstatement testing, before a definite conclusion can be reached as to whether the blockade of κ-opioid receptor-mediated anti-reward mechanisms represents a promising target for the treatment of alcohol addiction.

B. Translational Approach in Medication Development and New Clinical Trials

How can developments in preclinical medication research be translated to humans? In the field of research into medications for alcohol addiction, a roadmap for translational research has recently been provided by Markus Heilig and his research group at the NIAAA (154). Following their preclinical finding that mice genetically deficient in neurokinin 1 receptor show a marked decrease in voluntary alcohol consumption, the group performed an explorative randomized study in recently detoxified alcohol-dependent inpatients using the neurokinin 1 receptor antagonist LY686017 and placebo. LY686017 suppressed spontaneous alcohol craving, improved general well-being, blunted craving induced by a stress challenge procedure, and attenuated concomitant cortisol responses. In addition, it was shown that LY686017 reduced BOLD responses elicited by alcohol-related cues (154). These findings indicate the potential efficacy of this drug as an anti-craving and anti-relapse medication. This series of experiments represents a genuine translational approach to the linking of preclinical work and clinical efficacy, a

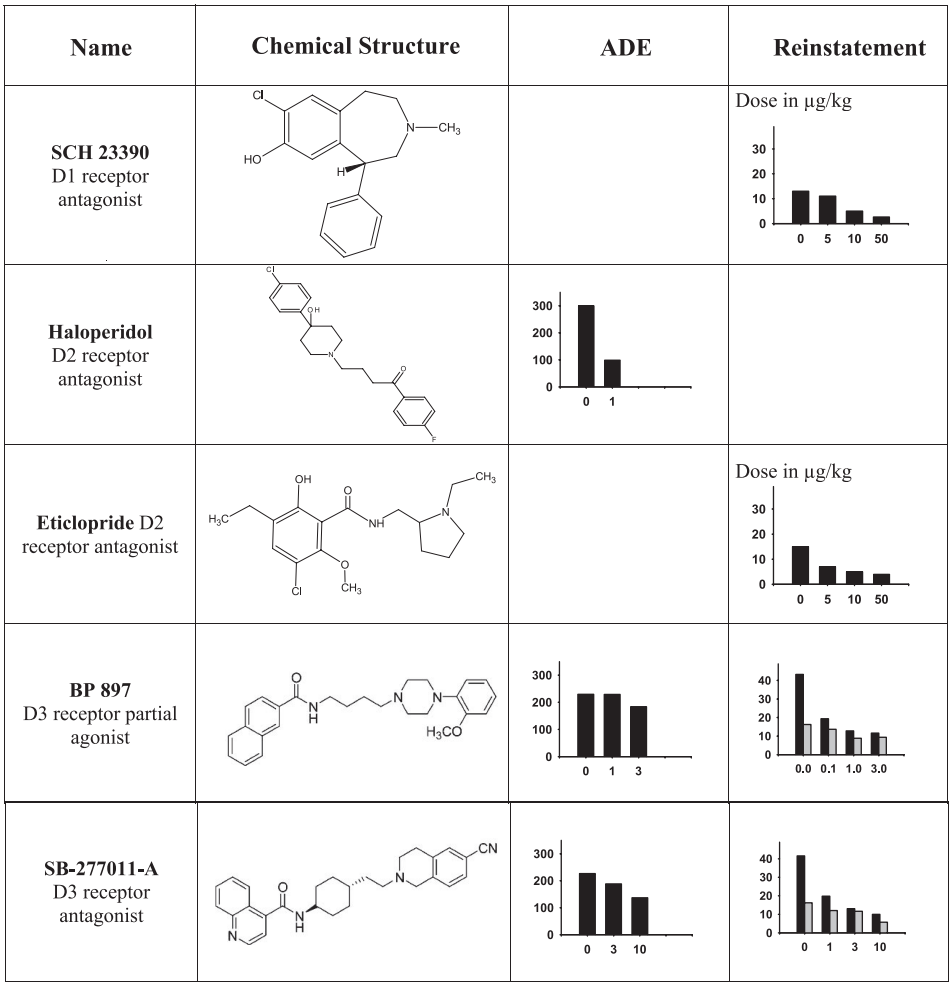


FIG. 14. Agents acting on dopamine receptors (details are as in Fig. 12); see Refs. 277, 420, 509. (Figure produced by Valentina Vengeliene.)

link which could otherwise only be established through the performance of time-consuming and cost-intensive phase II/III studies. Two pharmaceutical companies are now exploiting these positive results in full-scale clinical trials (319). This sets the example of how drug development should proceed, i.e., on the basis of the identification of putative target molecules from either a hypothesis-free whole genomic approach, or a transcriptomic approach. Functional validation must then be provided in appropriate animal models. Having achieved a positive signal in these animal models, studies in alcohol-dependent subjects need to be performed that include, as a minimum, measures of cue and stress reactivity. If a positive signal is once more obtained, then an RCT study is warranted.

Apart from LY686017, what other new clinical developments have occurred? Neramexane is a novel compound that has been classified as a moderate affinity, uncompetitive NMDA glutamate receptor antagonist. It exerts its effects by blocking the NMDA receptor channel, in a similar manner to the physiological channel blocker Mg^{2+}. Neramexane displays strong voltage dependency and a rapid blocking/unblocking kinetic. These pharmacological features allow neramexane to block the sustained activation of synaptic glutamate and to exit the receptor rapidly during normal physiological activation by millimolar concentrations of glutamate (360). Neramexane has yielded promising results in preclinical studies. In particular, it has been observed to reduce alcohol consumption following alcohol deprivation (198, 510), and a phase II study was recently initiated on the basis of these preclinical results. In this multicenter trial, neramexane was tested against placebo in detoxified alcohol-dependent subjects for the rate of continuous abstinence, duration of abstinence, craving, and drinking patterns. However, no major differences were detected between the two treatment groups for any of the outcome measures (G. A. Wiesbeck, personal communication). A reason for this lack of effect may have been the low doses administered. Relatively high doses of the drug should be administered in the context of its use as a substitution therapy, although this option is limited due to the relatively small therapeutic window of NMDA antagonists in alcohol-dependent subjects. Alterations in NMDA recep-

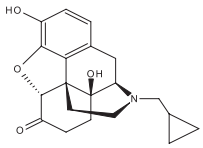
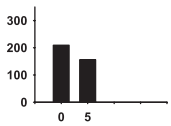
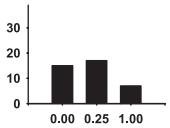
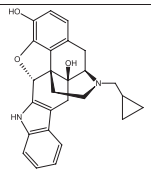
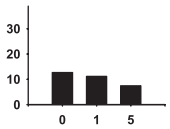
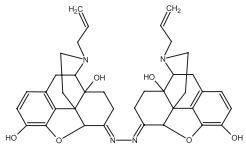
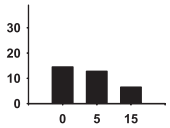
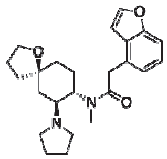
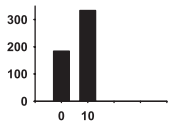
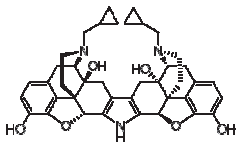
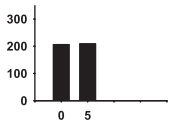
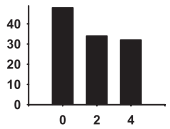
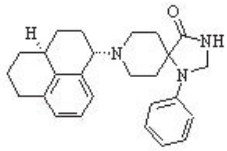
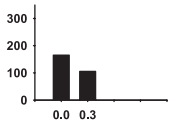
Name	Chemical Structure	ADE	Reinstatement
Naltrexone μ-opioid receptor antagonist			
Naltrindole δ-receptor antagonist			
Naloxonazine μ1-receptor antagonist			
CI-977 (enadoline) κ-receptor agonist		Dose in μg/kg/h 	
Nor-binaltorphimine (nor-BNI) κ-receptor antagonist			
Nociceptin (orphanin FQ) opioid-like nociceptin receptor endogenous ligand	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln		Dose in μg/kg, ICV 
Ro 64-6198 opioid-like nociceptin receptor agonist			

FIG. 15. Agents acting on opioid receptors (details are as in Fig. 12); see Refs. 82, 84, 197, 199, 257. (Figure produced by Valentina Vengeliene.)

tor subunit composition in alcohol-dependent subjects may also contribute to a lack of effect. NMDA receptors composed of NR1/NR3A subunits exhibit a reduced sensitivity to channel blockers compared with NR1/NR2A receptors (88). Importantly, alcohol-preferring msP rats have enhanced brain levels of NR3A and are almost insensitive to neramexane treatment (V. Vengeliene, unpublished data). Very high expression levels of NR3A are also found in the brains of psychiatric patients (331), underlining the conclusion that NMDA receptor channel blockers may only act as an effective substitution therapy in alcohol-dependent subjects when sufficient doses of these drugs are administered.

Topiramate (Topamax), an anticonvulsant compound that inhibits glutamate function and facilitates GABA function, reduces the harmful effects of excessive drinking as well as relapse rates in alcohol-dependent subjects (220). In a recently published study, continuously drinking alcohol-dependent subjects reached their goal of abstinence significantly quicker when treated with 300 mg/day topiramate compared with placebo (222).

Clinical studies indicate that baclofen, a stereoselective $GABA_B$ receptor agonist, may be a useful new drug in the treatment of patients with alcohol problems. Following promising findings from a pilot open study performed in a small sample of selected patients, the efficacy of

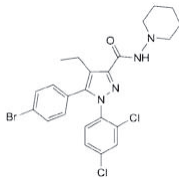
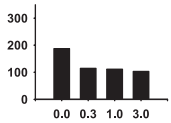
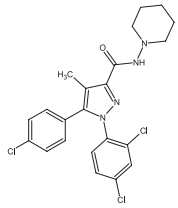
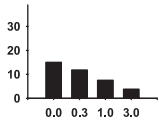
Name	Chemical Structure	ADE	Reinstatement
SR147778 CB1 receptor antagonist			
SR 141716 (A) (Rimonabant) CB1 receptor partial agonist			

FIG. 16. Agents acting on cannabinoid receptors (details are as in Fig. 12); see Refs. 86, 158. (Figure produced by Valentina Vengeliene.)

baclofen was recently evaluated in alcohol-dependent patients in a double-blind randomized controlled study (2). A significantly higher percentage of patients who achieved and maintained abstinence throughout the experimental period were found in the group treated with baclofen compared with the placebo group. Craving scores in the baclofen group were also consistently lower than those observed in the placebo group. In a recent study, the effectiveness and safety of baclofen in achieving and maintaining abstinence from alcohol in patients with liver cirrhosis was investigated. Of the patients allocated to baclofen, 70% achieved and maintained abstinence compared with 30% assigned to placebo. Cumulative abstinence duration was around twofold higher in patients allocated baclofen than in those assigned placebo. No hepatic side effects were recorded (3). Baclofen is effective in promoting abstinence from alcohol in alcohol-dependent patients with liver cirrhosis. The drug is well tolerated and may have an important role in the treatment of this patient group.

The 5-HT_3 antagonist ondansetron is another promising medication for the treatment of alcohol addiction. As outlined in section II*A*, the 5-HT_3 receptor is a primary site of action for the effects of ethanol in the brain. Following promising findings in animal work (Figs. 12–17), rigorous double-blind clinical studies were needed to test the efficacy of ondansetron in treating alcohol addiction. In a preliminary 6-wk double-blind clinical trial involving non-

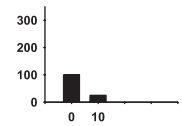
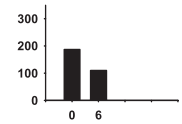
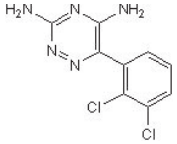
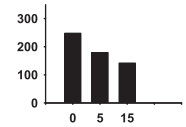
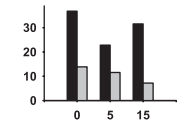
Name	Chemical Structure	ADE	Reinstatement
NPY neuropeptide Y	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2	Dose in μg/kg 	
Org 25935 GlyT-1 inhibitor	Not provided		
Lamotrigine voltage-gated Na+ channel blocker			

FIG. 17. Agents acting on other systems (details are as in Fig. 12); see Refs. 160, 325, 508. (Figure produced by Valentina Vengeliene.)

severely affected alcohol-dependent males, ondansetron was shown to be associated with a reduction in alcohol consumption (440). In a more recent large-scale, 12-wk double-blind, randomized controlled clinical trial, Johnson et al. (221) found that early-, but not late-onset alcohol-dependent men and women who received ondansetron had fewer drinks/day and drinks/drinking day compared with those given placebo. Ondansetron was more efficacious than placebo in increasing the percentage of abstinent days and total abstinent days per study week. More recently, Kranzler et al. (253) reported that ondansetron-treated early-onset alcohol-dependent subjects had significantly better drinking outcomes and fewer alcohol-related problems compared with their late-onset alcohol-dependent counterparts. The effect of ondansetron on cue-induced craving and NAC activation was also studied. A series of alcohol-related pictures, neutral beverage pictures, and visual control images were shown to alcohol-dependent subjects after a sip of alcohol following a 7-day period of double-blind randomly assigned daily dosing with ondansetron or placebo. Ondansetron decreased alcohol cue-induced activation of the NAC and craving (335). These results show that ondansetron is efficacious in the treatment of early-, but not late-onset alcoholism, as measured by improved drinking outcomes and decreased craving for alcohol.

Galantamine is a reversible, competitive inhibitor of acetylcholinesterase and is an allosteric modulator of nACh receptors (512). In the human brain, galantamine acts on the most abundant nACh receptor, the $\alpha4\beta2$ subtype (421). The activity of this subtype is thought to be particularly important since reduced activity of nACh receptors may contribute to decreased central cholinergic neurotransmission in alcohol-dependent patients. As outlined in section II, the nAch receptor-mediated acetylcholine/DA interaction may represent an important neurochemical access point in alcohol reinforcement. Furthermore, ethanol concentrations of $<$100 mM are known to potentiate $\alpha4\beta2$ subtypes of nACh receptors (178). This neurochemical interaction indicates the synergistic effects of alcohol and nicotine in reinforcement processes and provides a neurochemical correlate for the fact that alcohol drinking is strongly associated with smoking (272). This suggests that galantamine could be effective in prolonging abstinence in detoxified alcohol-dependent subjects. Mann et al. (294) investigated the efficacy and safety of galantamine in a 24-wk randomized, placebo-controlled, multicenter clinical trial in detoxified alcohol-dependent patients. Although galantamine did not extend the time to first severe relapse, additional post hoc analyses suggest that relapsed patients treated with galantamine consume less ethanol per drinking day than patients treated with placebo. This finding is in accordance with the proposed hypothesis that the blockade of nAch receptors should reduce alcohol reinforcement and thereby decrease overall alcohol consumption. Galanthamine could, therefore, play a role in reducing harmful use of alcohol and at-risk consumption. In the Mann et al. galanthamine trial, smoking behavior was also assessed by means of a patient diary. The nicotine metabolite cotinine was measured to verify the reported number of cigarettes smoked. Baseline smoking behavior did not differ between the galanthamine and placebo groups. Following treatment, significant differences were observed between the groups, with a 20% lower cumulative number of smoked cigarettes and a 15% lower number of smoking days in the galantamine group compared with the placebo group. The average number of cigarettes smoked per smoking day, as well as the cotinine values, decreased by ~10% (117). Galanthamine therefore provides a "double hit" on alcohol consumption and smoking and thus contributes significantly to harm reduction since almost all alcohol-dependent subjects smoke. Varenicline may similarly be administered to provide a "double hit" on alcohol drinking and smoking. Nicotine addiction is probably mediated through the activation of multiple nACh receptor subtypes, among which the mesolimbic $\alpha4\beta2$ plays a pivotal role. Partial agonists, which act on $\alpha4\beta2$ containing nAch receptors, have been designed as novel treatments for tobacco addiction. Such agents are thought to exert a dual effect by stimulating $\alpha4\beta2$-nACh receptor-mediated DA release sufficiently to reduce craving during abstinence and by inhibiting nicotine reinforcement during smoking (407). The validity of this dual approach has been demonstrated by the clinical efficacy of the $\alpha4\beta2$-nACh receptor partial agonist varenicline, which produces significantly better cessation rates than other treatments, and which thus represents a new option for smoking cessation pharmacotherapy (163, 224, 494). Varenicline has recently been investigated in several animal models of alcohol drinking. Acute administration of varenicline, in doses reported to reduce nicotine reinforcement, selectively reduced seeking for ethanol but not sucrose in an operant self-administration drinking paradigm. It also decreased voluntary consumption of alcohol but not water in animals chronically exposed to alcohol for 2 mo before varenicline treatment. Furthermore, chronic varenicline administration led to a decreased consumption of alcohol that did not result in a rebound increase in alcohol intake when varenicline was no longer administered (471). Considered together with the previous findings for galanthamine, these new findings suggest that varenicline might represent a new means of harm reduction for alcohol-dependent subjects, and appropriate clinical trials have already been initiated.

In conclusion, very promising compounds are on the horizon for both harm reduction and relapse prevention, with topiramate currently representing the most promising compound. Furthermore, a variety of novel compounds are currently being developed by pharmaceutical

companies, including D3 receptor antagonists, mGlu5 receptor antagonists, mGlu2/3 agonists, glycine transporter 1 blockers, CRHR1 antagonists, and novel CB1 antagonists (with the exception of rimonabant). Some of these compounds have already passed phase I and are soon to be tested in RCTs. The future therefore seems bright, and the pharmaceutical industry appears to have overcome its initial reluctance to become involved in this very lucrative market.

C. Individualized Pharmacotherapy for Alcoholism

Despite the efficacy of combined behavioral interventions and novel pharmacotherapies,[12] the maintenance of abstinence remains a challenge. Only around 20–30% of alcohol-dependent patients benefit from the available interventions, and therefore, it would be extremely helpful if responders to pharmacotherapy could be identified (319). An important step towards individualized medicine in the field of alcoholism would be the ability to identify acamprosate or naltrexone responders through the use of novel diagnostic biomarkers.

Response to pharmacological treatment may be influenced by genetic polymorphisms of drug target genes. It has recently been shown that a functional polymorphism in the μ-opioid receptor gene is associated with enhanced alcohol consumption in male rhesus macaques (26). The human equivalent of this gene variant (OPRM1A118G) predicts naltrexone efficacy as measured in terms of relapse behavior, with a large effect size being observed for naltrexone in OPRM1118G carriers, and no effect being detected in the majority of 118A homozygotes (12, 152, 351, 352). In this context, it is interesting that the alcohol-induced "high" is more blunted by naltrexone in OPRM1118G carriers than in 118A homozygotes among heavy alcohol drinkers (387).

In the future, it will be possible to apply such a pharmacogenetic approach to any medication that has a specific target gene (e.g., CRHR1). However, this will be more complicated when multiple target genes are involved in the treatment response, as is the case for acamprosate. Here, novel proteomic approaches may be more suitable for the development of biomarkers (528). The use of miniaturized and parallelized sandwich immunoassays, i.e., multi-analyte profiling, for instance, allows the accurate quantification of several hundred target proteins in human body fluids (485), and has already been successfully applied in the identification of biomarkers for a variety of disorders including depression and schizophrenia (77). With regard to the development of biomarkers for acamprosate response, proteomic profiling of the glutamate system may prove to be of interest, since acamprosate's mechanism of action seems to be due, at least in part, to a complex interaction with the glutamate system (295). A caveat, however, is how can alterations in the brain glutamate system be reflected in human body fluids? Recent studies have indicated that a good correlation (between 0.5–0.6) exists between gene expression profiles in blood and brain (1, 478), suggesting that protein markers have a similarly good correlation. Glutamate spectroscopy, as outlined in section VI*B*, may be an alternative to this proteomic approach in identifying acamprosate responders.

X. SUMMARY AND a PERSPECTIVE OF SYSTEMS-ORIENTED ALCOHOL RESEARCH

A. A Retrospective View of Neurobiological Alcohol Research

What have been the major achievements in neurobiologically oriented alcohol research? Some key publications have already been highlighted in the previous sections, and the following describes some other landmarks in alcohol research. In 1940, Curd Paul Richter (393) reported that laboratory rats voluntarily consume alcohol, although with high individual variability. This discovery marked the beginning of animal research in the study of alcohol. Furthermore, this observed variability in alcohol intake provided the basis for the generation of alcohol-preferring and nonpreferring rat and mouse lines, eight of which have been genetically selected since 1960 (137). Thousands of studies on alcohol drinking in rodents have been subsequently conducted, permitting the deciphering of the genetic and neurochemical basis of alcohol reinforcement. Studies of alcohol self-administration in laboratory animals remain crucial to the development of medication in the field of alcohol research; indeed, all available pharmacotherapies have been based on animal work of this nature.

Although not directly in conjunction with alcohol research, the discovery of the brain reinforcement/reward system in 1954 by James Olds (347), one of the outstanding experimental psychologists of the last century, ultimately provided the key to understanding the neuroanatomical correlates underlying alcohol reinforcement. The foundations for understanding the neurochemical substrates of alcohol reward were laid in 1973 by the three

[12] In the large-scale study COMBINE (11), over 1,300 patients were treated with either naltrexone or placebo. While half the patients received a low-dose standard supportive therapy (Medical Management), the other half received a more intensive psychotherapy, i.e., cognitive-behavioral intervention (CBI). All groups showed a substantial reduction in drinking. During treatment, those patients receiving naltrexone plus medical management, CBI plus medical management and placebo, or both naltrexone and CBI plus medical management had a higher percentage of abstinent days than the group receiving placebo and medical management only, which is indicative of a significant naltrexone × behavioral intervention interaction.

research teams responsible for identifying the first opioid receptors (367, 447, 486). Only two years later in the hunt for the endogenous ligands, John Hughes and Hans Kosterlitz (206) identified the first opioids in the brain and called them enkephalins. These findings not only promoted opioid research in general, but also represented key discoveries for subsequent alcohol research. Endogenous opioid systems are thought to induce the pleasurable and rewarding effects of alcohol, and thereby constitute ideal targets for treatment. The first description of opioid receptor blockade by means of naltrexone, and the resultant reduction of voluntary alcohol consumption in rats (9), marked the starting point of the development of relapse medication in alcohol research. A decade later, the first reports on the clinical efficacy of naltrexone in alcohol-dependent patients were published (349, 517), and a recent meta-analysis of 24 randomized RCTs that included a total of 2,861 subjects demonstrates that naltrexone decreases the relative risk of relapse compared with placebo by a significant 36% (470). A further milestone in medication development was the finding that a functional polymorphism of the μ-opioid receptor gene may predict response to naltrexone (351). Although this finding has recently been replicated (12), no final judgement on this pharmacogenetic discovery will be possible for several years. Nevertheless, given the fact that our century is dominated by the belief that personalized medicine will power further biomedical developments, the study of Oslin et al. (351) has already marked this shift in paradigms. Despite the promise of pharmacogenetics in identifying treatment responders, there have, to date, been very few success stories in any aspect of medicine.

B. A Summary of the Present Review

The structure of this review follows a systems approach towards achieving a better understanding of the acute and chronic effects of alcohol. The interaction of the ethanol molecule at all system levels has been reviewed in detail, and this section highlights the key points.

The first level of interaction concerns the primary targets of ethanol in the brain. Ethanol has only a few primary targets, and these include the NMDA, $GABA_A$, glycine, 5-HT_3, and nACh receptors, as well as L-type Ca^{2+} channels and G protein-activated inwardly rectifying K^+ channels. Following the initial ethanol effect on these receptors and ion channels, a second wave of indirect effects on monoamines, opioids, and endocannabinoids then occurs that is crucial for the initiation of alcohol reinforcement and reward.

The primary and secondary effects of ethanol involve both PKA and PKC signaling. Activation of PKA signaling is the consequence of acute exposure to alcohol, whereas chronic alcohol exposure leads to an adaptive downregulation of this pathway, in particular of CREB function. In addition, PKA signaling in medium spiny neurons affects DARPP-32 function, which is an important regulator of NMDA receptor activity within the reinforcement system and which may therefore play an important role in neuroadaptation in response to chronic alcohol exposure. NMDA receptors are closely linked to NO/cGMP signaling, and this pathway also plays a critical role in mediating alcohol reinforcement. PKC signaling is significantly affected by ethanol, which, in turn, affects $GABA_A$ receptor function. Alcohol therefore affects the activity of receptors relevant to synaptic plasticity (i.e., glutamate and GABA receptors), as well as influencing CREB-mediated processes.

Altered CREB function affects multiple alcohol-responsive target genes, the most prominent being CRH, prodynorphin, BDNF, and NPY. Other, mainly CREB-independent, alcohol-responsive genes have been identified by means of microarray analysis, and more than 50 genes mainly related to neurotransmission and signal transduction have now been functionally validated as being critically involved in alcohol reinforcement processes.

It has been proposed that persistent alcohol-induced alterations in gene expression may underlie enduring adaptations and maladaptations in the brain, thus defining the irreversible transition from controlled to compulsive drug use. Such persistent alterations have not yet been identified. It has been alternatively proposed that epigenetic mechanisms, which exert an enduring control over gene expression without altering the genetic code, may mediate persistent molecular alterations within the reinforcement system. Elevated genomic DNA methylation and acetylation, which lead to altered global gene expression, are indeed found following chronic alcohol exposure. The alteration in DNA methylation in the promoter regions of α-synuclein exemplifies such maladaptive molecular responses to chronic alcohol that may have lasting effects on DA-dependent alcohol seeking.

Studies investigating neuronal network activity using neuroimaging techniques in humans have yielded useful information regarding the neuroanatomical and neurochemical substrates of addictive behavior. In the "addicted brain," this research has indicated the involvement of the extended amygdala, including the NAC, the orbitofrontal cortex, and the dorsal striatum, brain areas responsible for reinforcement, decision-making, and impulse control. Hypofunction of the DAergic system and alterations within endogenous opioid systems appear to correlate with craving and relapse behavior. Molecules involved in endocrine HPA activity and the regulation of emotion by the amygdala, such as CRH and NPY, ultimately mediate environmental influences on addictive behavior. Despite these advances in knowledge, our understanding of the molecular and physiological nature of addictive behavior remains poor.

C. A Perspective of Systems-Oriented Alcohol Research

Neurobiologically driven research clearly indicates that the development of a complex psychiatric disorder such as alcoholism is not caused by any single gene or simple molecular event. However, the reductionist research approach only permits testing of the involvement of a single gene or a simple molecular event in the etiology of alcoholism. This dilemma can only be solved by the application of a systems biology approach. This necessitates the breaking down of a system into different levels, as exemplified by the structure of this review. The different levels can then be studied using new -omics technologies which allow the identification of genetic variations and quantification of molecules at the level of mRNA, protein, and metabolites. Furthermore, the use of multielectrode in vivo recordings enables us to learn more about the neuronal network alterations involved in disease progression, while a variety of neuroimaging techniques allow the evaluation of neuronal network activity on a much larger scale. For the first time, therefore, we are in a position to gather comprehensive data systematically on different biological system levels. In such a hypothesis-free approach, we receive bioinformation on all system levels, ranging from the gene to molecules to synaptic plasticity to neuronal network activity. Although information from genome-wide association studies and proteomics is still lacking at this time, data derived from QTL analysis and other genetic research together with large-scale gene expression profiling have already been successful in defining new clusters of genes involved in mediating the acute and chronic effects of alcohol. By means of computational neuroscience, this novel information can be combined with what has been learned during the 30 years' experience of a hypothesis-driven reductionist approach in neurobiologically oriented alcohol research, and this will then hopefully lead to a better understanding of the molecular and physiological processes underlying alcoholism.

ACKNOWLEDGMENTS

I thank Wolfgang Sommer, Stefan Bleich, Bernd Lenz, Peter Gebicke-Haerter, Gabi Ende, Ainhoa Bilbao, and Miriam Schneider for helpful comments on the manuscript.

Address for reprint requests and other correspondence: R. Spanagel, Dept. of Psychopharmacology, Central Institute of Mental Health, Univ. of Heidelberg, J5, 68159 Mannheim, Germany (e-mail: rainer.spanagel@zi-mannheim.de).

GRANTS

The author's research is supported by several EU (IMAGEN, PHECOMP, ERAP), BMBF (SUFO-BW, NGFN), and DFG (SFB636, SP383) grants.

REFERENCES

1. **Achiron A, Gurevich M.** Peripheral blood gene expression signature mirrors central nervous system disease. *Autoimmun Rev* 5: 517–522, 2006.
2. **Addolorato G, Abenavoli L, Leggio L, DeLorenzi G, Ferrulli A, Caputo F, Agabio R, Gessa GL, Colombo G, Gasbarrini G.** Baclofen: clinical data. In: *Drugs for Relapse Prevention of Alcoholism*. edited by Spanagel R, Mann K. Basel: Birkhäuser, 2005, p. 170–193.
3. **Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonghia L, Mirijello A, Abenavoli L, D'Angelo C, Caputo F, Zambon A, Haber PS, Gasbarrini G.** Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-blind controlled study. *Lancet* 370: 1915–1922, 2007.
4. **Ahlenius S, Carlsson A, Engel J, Svensson T, Södersten P.** Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin Pharmacol Ther* 14: 586–591, 1973.
5. **Ahn AC, Tewari M, Poon CS, Phillips RS.** The clinical applications of a systems approach. *PLoS Medicine* 3: e209, 2007.
6. **Albrecht U, Eichele G.** The mammalian circadian clock. *Curr Opin Genet Dev* 13: 271–277, 2003.
7. **Almeida OF, Shoaib M, Deicke J, Fischer D, Darwish MH, Patchev VK.** Gender differences in ethanol preference and ingestion in rats. The role of the gonadal steroid environment. *J Clin Invest* 101: 2677–2685, 1998.
8. **Althausen S, Paschen W.** Homocysteine-induced changes in mRNA levels of genes coding for cytoplasmic- and endoplasmic reticulum-resident stress proteins in neuronal cell cultures. *Brain Res* 84: 32–40, 2000.
9. **Altshuler HL, Phillips PE, Feinhandler DA.** Alteration of ethanol self-administration by naltrexone. *Life Sci* 26: 679–688, 1980.
10. **Angst J, Sellaro R, Ries Merikangas K.** Multimorbidity of psychiatric disorders as an indicator of clinical severity. *Eur Arch Psychiatry Clin Neurosci* 252: 147–154, 2002.
11. **Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, LoCastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A.** COMBINE Study Research Group. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. *JAMA* 295: 2003–2017, 2006.
12. **Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D.** An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. *Arch Gen Psychiatry* 65: 135–144, 2008.
13. **Aragon CM, Stotland LM, Amit Z.** Studies on ethanol-brain catalase interaction: evidence for central ethanol oxidation. *Alcohol Clin Exp Res* 15: 165–169, 1991.
14. **Arlinde C, Sommer W, Bjork K, Reimers M, Hyytia P, Kiianmaa K, Heilig M.** A cluster of differentially expressed signal transduction genes identified by microarray analysis in a rat genetic model of alcoholism. *Pharmacogenomics J* 4: 208–218, 2004.
15. **Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, Le Fur G.** Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology* 132: 104–106, 1997.
16. **Asyyed A, Storm D, Diamond I.** Ethanol activates cAMP response element-mediated gene expression in select regions of the mouse brain. *Brain Res* 1106: 63–71, 2006.
17. **Bachteler D, Economidou D, Danysz W, Ciccocioppo R, Spanagel R.** The effects of acamprosate and neramexane on cue-induced reinstatement of ethanol-seeking behavior in rat. *Neuropsychopharmacology* 30: 1104–1110, 2005.
18. **Bäckström P, Bachteler D, Koch S, Hyytiä P, Spanagel R.** mGluR5 antagonist MPEP reduces ethanol-seeking and relapse behavior. *Neuropsychopharmacology* 29: 921–928, 2004.

19. **Bäckstrom P, Hyytiä P.** Ionotropic glutamate receptor modulate cue-induced reinstatement of ethanol-seeking behavior. *Alcohol Clin Exp Res* 28: 558–565, 2004.
20. **Bäckstrom P, Hyytiä P.** Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG. *Eur J Pharmacol* 528: 110–118, 2005.
21. **Baldo BA, Daniel RA, Berridge CW, Kelley AE.** Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *J Comp Neurol* 464: 220–237, 2003.
22. **Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS.** Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. *J Pharmacol Exp Ther* 264: 489–495, 1993.
23. **Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ.** International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev* 50: 291–313, 1998.
24. **Barr CS, Schwandt ML, Newman TK, Higley JD.** The use of adolescent nonhuman primates to model human alcohol intake: neurobiological, genetic, and psychological variables. *Ann NY Acad Sci* 1021: 221–233, 2004.
25. **Barr CS, Newman TK, Lindell S, Shannon C, Champoux M, Lesch KP, Suomi SJ, Goldman D, Higley JD.** Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Arch Gen Psychiatry* 61: 1146–1152, 2004.
26. **Barr CS, Schwandt M, Lindell SG, Chen SA, Goldman D, Suomi SJ, Higley JD, Heilig M.** Association of a functional polymorphism in the mu-opioid receptor gene with alcohol response and consumption in male rhesus macaques. *Arch Gen Psychiatry* 64: 369–376, 2007.
27. **Baur JA, Sinclair DA.** Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* 5: 493–506, 2006.
28. **Bayerlein K, Hillemacher T, Reulbach U, Mugele B, Sperling W, Kornhuber J, Bleich S.** Alcoholism-associated hyperhomocysteinemia and previous withdrawal seizures. *Biol Psychiatry* 57: 1590–1593, 2005.
29. **Becker HC.** Positive relationship between the number of prior ethanol withdrawal episodes and the severity of subsequent withdrawal seizures. *Psychopharmacology* 116: 26–32, 1994.
30. **Belin D, Everitt BJ.** Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron* 57: 432–441, 2008.
31. **Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ.** The alcohol-preferring P rat and animal models of excessive alcohol drinking. *Addict Biol* 11: 270–288, 2006.
32. **Bellone C, Luscher C.** Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat Neurosci* 9: 636–641, 2006.
33. **Belluzzi JD, Stein L.** Enkephalin may mediate euphoria and drive-reduction reward. *Nature* 266: 556–558, 1977.
34. **Bendszus M, Weijers HG, Wiesbeck G.** Sequential MR imaging and proton MR spectroscopy in patients who underwent recent detoxification for chronic alcoholism: correlation with clinical and neuropsychological data. *AJNR Am J Neuroradiol* 22: 1926–1932, 2001.
35. **Benegal V, Antony G, Venkatasubramanian G, Jayakumar PN.** Gray matter volume abnormalities and externalizing symptoms in subjects at high risk for alcohol dependence. *Addict Biol* 12: 122–32, 2007.
36. **Besancon F.** Time to alcohol dependence after abstinence and first drink. *Addiction* 88: 1647–1650, 1993.
37. **Bestor TH.** The DNA methyltransferases of mammals. *Hum Mol Genet* 9: 2395–2402, 2000.
38. **Bhave SV, Hoffman PL, Lassen N, Vasiliou V, Saba L, Deitrich RA, Tabakoff B.** Gene array profiles of alcohol and aldehyde metabolizing enzymes in brains of C57BL/6 and DBA/2 mice. *Alcohol Clin Exp Res* 30: 1659–1669, 2006.
39. **Biegon A, Gibbs A, Alvarado M, Ono M, Taylor S.** In vitro and in vivo characterization of [^{3}H]CNS-5161—a use-dependent ligand for the *N*-methyl-D-aspartate receptor in rat brain. *Synapse* 61: 577–586, 2007.
40. **Bienkowski P, Koros E, Kostowski W, Danysz W.** Effects of *N*-methyl-D-aspartate receptor antagonists on reinforced and non-reinforced responding for ethanol in rats. *Alcohol* 18: 131–137, 1999.
41. **Birney E.** ENCODE Project consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447: 799–816, 2007.
42. **Björk K, Saarikoski ST, Arlinde C, Kovanen L, Osei-Hyiaman D, Ubaldi M, Reimers M, Hyytia P, Heilig M, Sommer WH.** Glutathione-*S*-transferase expression in the brain: possible role in ethanol preference and longevity. *FASEB J* 20: 1826–1835, 2006.
43. **Björk K, Rimondini R, Hansson AC, Terasmaa A, Hyytia P, Heilig M, Sommer WH.** Modulation of voluntary ethanol consumption by beta-arrestin 2. *FASEB J* 22: 2552–2560, 2008.
44. **Blaha CD, Yang CR, Floresco SB, Barr AM, Phillips AG.** Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. *Eur J Neurosci* 9: 902–911, 1997.
45. **Blednov YA, Walker D, Martinez M, Harris RA.** Reduced alcohol consumption in mice lacking preprodynorphin. *Alcohol* 40: 73–86, 2006.
46. **Bleich S, Bleich K, Kropp S, Bittermann HJ, Degner D, Sperling W, Rüther E, Kornhuber J.** Moderate alcohol consumption in social drinkers raises plasma homocysteine levels: a contradiction to the "French Paradox"? *Alcohol Alcohol* 36: 189–192, 2001.
47. **Bleich S, Carl M, Bayerlein K, Reulbach U, Biermann T, Hillemacher T, Bönsch D, Kornhuber J.** Evidence of increased homocysteine levels in alcoholism: the Franconian alcoholism research studies (FARS). *Alcohol Clin Exp Res* 29: 334–336, 2005.
48. **Blomeyer D, Treutlein J, Esser G, Schmidt MH, Schumann G, Laucht M.** Interaction between CRHR1 gene and stressful life events predicts adolescent heavy alcohol use. *Biol Psychiatry* 63: 146–151, 2008.
49. **Blomqvist O, Engel JA, Nissbrandt H, Söderpalm B.** The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *Eur J Pharmacol* 249: 207–213, 1993.
50. **Blomqvist O, Ericson M, Engel JA, Söderpalm B.** Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. *Eur J Pharmacol* 334: 149–156, 1997.
51. **Blood AJ, Zatorre RJ.** Intensely pleasurable responses to music correlate with activity in brain regions implicated in reward and emotion. *Proc Natl Acad Sci USA* 98: 11818–11823, 2001.
52. **Bloom F, Battenberg E, Rossier J, Ling N, Guillemin R.** Neurons containing β-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. *Proc Natl Acad Sci USA* 75: 1591–1595, 1978.
53. **Boehm SL 2nd, Ponomarev I, Jennings AW, Whiting PJ, Rosahl TW, Garrett EM, Blednov YA, Harris RA.** γ-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. *Biochem Pharmacol* 68: 1581–1602, 2004.
54. **Boettiger CA, Mitchell JM, Tavares VC, Robertson M, Joslyn G, D'Esposito M, Fields HL.** Immediate reward bias in humans: fronto-parietal networks and a role for the catechol-*O*-methyltransferase 158(Val/Val) genotype. *J Neurosci* 27: 14383–14391, 2007.
55. **Bönsch D, Greifenberg V, Bayerlein K, Biermann T, Reulbach U, Hillemacher T, Kornhuber J, Bleich S.** Alpha-synuclein protein levels are increased in alcoholic patients and are linked to craving. *Alcohol Clin Exp Res* 29: 763–765, 2005.
56. **Bönsch D, Lenz B, Kornhuber J, Bleich S.** DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. *Neuroreport* 16: 167–170, 2005.
57. **Bönsch D, Lenz B, Reulbach U, Kornhuber J, Bleich S.** Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. *J Neural Transm* 111: 1611–1616, 2004.
58. **Bönsch D, Reulbach U, Bayerlein K, Hillemacher T, Kornhuber J, Bleich S.** Elevated alpha synuclein mRNA levels are associated with craving in patients with alcoholism. *Biol Psychiatry* 56: 984–986, 2004.
59. **Bohman M, Cloninger R, Sigvardsson S, von Knorring AL.** The genetics of alcoholisms and related disorders. *J Psychiatr Res* 21: 447–452, 1987.

60. **Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A.** Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 49: 226–231, 2003.
61. **Bolo N, Nedelec JF, Muzet M, De Witte P, Dahchour A, Durbin P, Macher JP.** Central effects of acamprosate: part 2. Acamprosate modifies the brain in-vivo proton magnetic resonance spectrum in healthy young male volunteers. *Psychiatry Res* 82: 115–127, 1998.
62. **Boweres BJ, Wehner JM.** Ethanol consumption and behavioral impulsivity are increased in protein kinase Cgamma null mutant mice. *J Neurosci* 21: RC180, 2001.
63. **Bredt DS, Snyder SH.** Nitric oxide: a physiologic messenger molecule. *Annu Rev Biochem* 6: 175–195, 1994.
64. **Breese GR, Overstreet DH, Knapp DJ.** Conceptual framework for the etiology of alcoholism: a "kindling"/stress hypothesis. *Psychopharmacology* 178: 367–380, 2005.
65. **Bucci BK, Kruse SW, Thode AB, Alvardo SM, Jones DN.** Effect of *n*-alcohols on the structure and stability of the *Drosophila* odorant binding protein LUSH. *Biochemistry* 45: 1693–1701, 2006.
66. **Burish TG, Maisto SA, Cooper AM, Sobell MB.** Effects of voluntary short-term abstinence from alcohol on subsequent drinking patterns of college students. *J Stud Alcohol* 42: 1013–1120, 1981.
67. **Caillé S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH.** Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J Neurosci* 27: 3695–3702, 2007.
68. **Calapai G, Mazzaglia G, Sautebin L, Costantino G, Marciano MC, Cuzzocrea S, Di R, Caputi AP.** Inhibition of nitric oxide formation reduces voluntary ethanol consumption in the rat. *Psychopharmacology* 125: 398–401, 1996.
69. **Campbell AD, Kohl RR, McBride WJ.** Serotonin-3 receptor and ethanol-stimulated somatodendritic dopamine release. *Alcohol* 13: 569–574, 1996.
70. **Carai MAM, Agabio R, Addolorato G, Gessa GL, Colombo G.** Baclofen: preclinical data. In: *Drugs for Relapse Prevention of Alcoholism*, edited by Spanagel R, Mann K. Basel: Birkhäuser, 2005, p. 163–170.
71. **Carboni E, Acquas E, Frau R, Di Chiara G.** Differential inhibitory effects of a 5-HT_3 antagonist on drug-induced stimulation of dopamine release. *Eur J Pharmacol* 164: 515–519, 1989.
72. **Carlezon WA Jr, Duman RS, Nestler EJ.** The many faces of CREB. *Trends Neurosci* 28: 436–445, 2005.
73. **Carr LG, Spence JP, Peter Eriksson CJ, Lumeng L, Li Colombo G TK, Lobina C, Carai MA, Gessa GL.** Phenotypic characterization of genetically selected Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats. *Addict Biol* 11: 324–338, 2006.
74. **Carr LG, Spence JP, Peter Eriksson CJ, Lumeng L, Li TK.** AA and ANA rats exhibit the R100Q mutation in the $GABA_A$ receptor alpha 6 subunit. *Alcohol* 31: 93–97, 2003.
75. **Castaneda R, Sussman N, Westreich L, Levy R, O'Malley M.** A review of the effects of moderate alcohol intake on the treatment of anxiety and mood disorders. *J Clin Psychiatry* 57: 207–212, 1996.
76. **Chakir M, Peridy O, Capy P, Pla E, David JR.** Adaptation to alcoholic fermentation in *Drosophila*: a parallel selection imposed by environmental ethanol and acetic acid. *Proc Natl Acad Sci USA* 90: 3621–3625, 1993.
77. **Chan SM, Ermann J, Su L, Fathman CG, Utz PJ.** Protein microarrays for multiplex analysis of signal transduction pathways. *Nat Med* 10: 1390–1396, 2005.
78. **Chen CP, Kuhn P, Advis JP, Sarkar DK.** Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat. *J Neurochem* 88: 1547–1554, 2004.
79. **Chen CP, Kuhn P, Advis JP, Sarkar DK.** Prenatal ethanol exposure alters the expression of period genes governing the circadian function of beta-endorphin neurons in the hypothalamus. *J Neurochem* 97: 1026–1033, 2006.
80. **Choi SJ, Kim KJ, Cho HS, Kim SY, Cho YJ, Hahn SJ, Sung KW.** Acute inhibition of corticostriatal synaptic transmission in the rat dorsal striatum by ethanol. *Alcohol* 40: 95–101, 2006.
81. **Choi DS, Wang D, Dadgar J, Chang WS, Messing RO.** Conditional rescue of protein kinase C epsilon regulates ethanol preference and hypnotic sensitivity in adult mice. *J Neurosci* 22: 9905–9911, 2002.
82. **Ciccocioppo R, Martin-Fardon R, Weiss F.** Effect of selective blockade of mu(1) or delta opioid receptors on reinstatement of alcohol-seeking behavior by drug-associated stimuli in rats. *Neuropsychopharmacology* 27: 391–399, 2002.
83. **Ciccocioppo R, Economidou D, Cippitelli A, Cucculelli M, Ubaldi M, Soverchia L, Lourdusamy A, Massi M.** Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism. *Addict Biol* 11: 339–355, 2006.
84. **Ciccocioppo R, Economidou D, Fedeli A, Angeletti S, Weiss F, Heilig M, Massi M.** Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/orphanin FQ in alcohol-preferring rats. *Psychopharmacology* 172: 170–178, 2004.
85. **Ciccocioppo R, Panocka I, Froldi R, Colombo G, Gessa GL, Massi M.** Antidepressant-like effect of ethanol revealed in the forced swimming test in Sardinian alcohol-preferring rats. *Psychopharmacology* 144: 151–157, 1999.
86. **Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M, Massi M, Bermudez-Silva FJ, Navarro M, Ciccocioppo R, de Fonseca FR.** The European TARGALC Consortium. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur J Neurosci* 21: 2243–2251, 2005.
87. **Civelli O, Bunzow JR, Grandy DK.** Molecular diversity of dopamine receptors. *Annu Rev Pharmacol Toxicol* 32: 281–307, 1993.
88. **Chatterton JE, Awobuluyi M, Premkumar LS, Takahashi H, Talantova M, Shin Y, Cui J, Tu S, Sevarino KA, Nakanishi N, Tong G, Lipton SA, Zhang D.** Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* 415: 793–798, 2002.
89. **Clarke TK, Treutlein J, Zimmermann US, Kiefer F, Skowronek MH, Rietschel M, Mann K, Schumann G.** HPA-axis activity in alcoholism: examples for a gene-environment interaction. *Addict Biol* 13: 1–14, 2008.
90. **Cloninger CR.** Neurogenetic adaptive mechanisms in alcoholism. *Science* 236: 410–416, 1987.
91. **Cohen C, Perrault G, Sanger DJ.** Preferential involvement of D3 versus D2 dopamine receptors in the effects of dopamine receptor ligands on oral ethanol self-administration in rats. *Psychopharmacology* 140: 478–485, 1998.
92. **Colombo G, Grant KA.** NMDA receptor complex antagonists have ethanol-like discriminative stimulus effects. *Ann NY Acad Sci* 654: 421–423, 1992.
93. **Colombo G, Lobina C, Carai MA, Gessa GL.** Phenotypic characterization of genetically selected Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats. *Addict Biol* 11: 324–338, 2006.
94. **Colombo G, Serra S, Brunetti G, Gomez R, Melis S, Vacca G, Carai MM, Gessa L.** Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology* 159: 181–187, 2002.
95. **Colombo G, Serra S, Brunetti G, Vacca G, Carai MA, Gessa GL.** Suppression by baclofen of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. *Drug Alcohol Depend* 70: 105–108, 2003.
96. **Colombo G, Serra S, Vacca G, Carai MA, Gessa GL.** Endocannabinoid system and alcohol addiction: pharmacological studies. *Pharmacol Biochem Behav* 81: 369–380, 2005.
97. **Conger JJ.** Reinforcement theory and the dynamics of alcoholism. *Q J Stud Alcohol* 18: 296–305, 1956.
98. **Corder R, Douthwaite JA, Lees DM, Khan NQ, Viseu Dos Santos AC, Wood EG, Carrier MJ.** Endothelin-1 synthesis reduced by red wine. *Nature* 414: 863–864, 2001.
99. **Corder R, Mullen W, Khan NQ, Marks SC, Wood EG, Carrier MJ, Crozier A.** Oenology: red wine procyanidins and vascular health. *Nature* 444: 566, 2006.
100. **Covarrubias MY, Khan RL, Vadigepalli R, Hoek JB, Schwaber JS.** Chronic alcohol exposure alters transcription broadly in a key integrative brain nucleus for homeostasis: the nucleus tractus solitarius. *Physiol Gen* 24: 45–58, 2005.

101. **Cowen MS, Schroff KC, Gass P, Sprengel R, Spanagel R.** Neurobehavioral effects of alcohol in AMPA receptor subunit (GluR1) deficient mice. *Neuropharmacology* 45: 325–333, 2003.
102. **Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF.** Alcohol-related genes: contributions from studies with genetically engineered mice. *Addict Biol* 11: 195–269, 2006.
103. **Crawford DK, Trudell JR, Bertaccini EJ, Li K, Davies DL, Alkana RL.** Evidence that ethanol acts on a target in Loop 2 of the extracellular domain of alpha1 glycine receptors. *J Neurochem* 102: 2097–2109, 2007.
104. **Crews FT, Braun CJ, Hoplight B, Switzer RC 3rd, Knapp DJ.** Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. *Alcohol Clin Exp Res* 24: 1712–1723, 2000.
105. **Cummings DE, Naleid AM, Figlewicz Lattemann DP.** Ghrelin: a link between energy homeostasis and drug abuse? *Addict Biol* 12: 1–5, 2007.
106. **Dahchour A, De Witte P.** Effects of acamprosate on excitatory amino acids during multiple ethanol withdrawal periods. *Alcohol Clin Exp Res* 27: 465–470, 2003.
107. **Dahchour A, De Witte P, Bolo N, Nedelec JF, Muzet M, Durbin P, Macher JP.** Central effects of acamprosate: part 1. Acamprosate blocks the glutamate increase in the nucleus accumbens microdialysate in ethanol withdrawn rats. *Psychiatry Res* 82: 107–114, 1998.
108. **Daniels GM, Buck KJ.** Expression profiling identifies strain-specific changes associated with ethanol withdrawal in mice. *Genes Brain Behav* 1: 35–45, 2002.
109. **Davidson KM, Ritson EB.** The relationship between alcohol dependence and depression. *Alcohol Alcohol* 28: 147–155, 1993.
110. **Dayas CV, McGranahan TM, Martin-Fardon R, Weiss F.** Stimuli linked to ethanol availability activate hypothalamic CART and orexin neurons in a reinstatement model of relapse. *Biol Psychiatry* 63: 152–157, 2008.
111. **Devine DP, Wise RA.** Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci* 14: 1978–1984, 1994.
112. **Dawson DA, Grant BF, Ruan WJ.** The association between stress and drinking: modifying effects of gender and vulnerability. *Alcohol Alcohol* 40: 453–460, 2005.
113. **Detich N, Hamm S, Just G, Knox JD, Szyf M.** The methyl donor *S*-adenosylmethionine inhibits active demethylation of DNA: a candidate novel mechanism for the pharmacological effects of *S*-adenosylmethionine. *J Biol Chem* 278: 20812–20820, 2003.
114. **Diamond I, Gordon AS.** Cellular and molecular neuroscience of alcoholism. *Physiol Rev* 77: 1–19, 1997.
115. **Di Chiara G, Imperato A.** Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85: 5274–5278, 1988.
116. **Dickinson A, Wood N, Smith JW.** Alcohol seeking by rats: action or habit? *Q J Exp Psychol* 55: 331–348, 2002.
117. **Diehl A, Nakovics H, Croissant B, Smolka MN, Batra A, Mann K.** Galantamine reduces smoking in alcohol-dependent patients: a randomized, placebo-controlled trial. *Int J Clin Pharmacol Ther* 44: 614–622, 2006.
118. **Dixit AR, Crum RM.** Prospective study of depression and the risk of heavy alcohol use in women. *Am J Psychiatry* 157: 751–758, 2000.
119. **Dong Y, Saal D, Thomas M, Faust R, Bonci A, Robinson T, Malenka RC.** Cocaine-induced potentiation of synaptic strength in dopamine neurons: behavioral correlates in GluRA(−/−) mice. *Proc Natl Acad Sci USA* 101: 14282–14287, 2004.
120. **Doty P, de Wit H.** Effects of naltrexone pretreatment on the subjective and performance effects of ethanol in social drinkers. *Behav Pharmacol* 6: 386–394, 1995.
121. **Doty P, Kirk JM, Cramblett MJ, de Wit H.** Behavioral responses to ethanol in light and moderate social drinkers following naltrexone pretreatment. *Drug Alcohol Depend* 47: 109–116, 1997.
122. **Drobes DJ, Anton RF, Thomas SE, Voronin K.** Effects of naltrexone and nalmefene on subjective response to alcohol among non-treatment-seeking alcoholics and social drinkers. *Alcohol Clin Exp Res* 28: 1362–1370, 2004.
123. **Dudley R.** Evolutionary origins of human alcoholism in primate frugivory. *Q Rev Biol* 75: 3–15, 2000.
124. **Duka T, Gentry J, Malcolm R, Ripley TL, Borlikova G, Stephens DN, Veatch LM, Becker HC, Crews FT.** Consequences of multiple withdrawals from alcohol. *Alcohol Clin Exp Res* 28: 233–246, 2004.
125. **Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM.** Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. *Alcohol* 10: 207–212, 1993.
126. **Economidou D, Hansson AC, Weiss F, Terasmaa A, Sommer WH, Cippitelli A, Fedeli A, Martin-Fardon R, Massi M, Ciccocioppo R, Heilig M.** Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. *Biol Psychiatry* 64: 211–218, 2008.
127. **Edenberg HJ, Foroud T.** The genetics of alcoholism: identifying specific genes through family studies. *Addict Biol* 11: 386–396, 2006.
128. **Egger G, Liang G, Aparicio A, Jones PA.** Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429: 457–463, 2004.
129. **Ende G, Walter S, Welzel H, Demirakca T, Wokrina T, Ruf M, Ulrich M, Diehl A, Henn FA, Mann K.** Alcohol consumption significantly influences the MR signal of frontal choline-containing compounds. *Neuroimage* 32: 740–746, 2006.
130. **Ende G, Welzel H, Walter S, Weber-Fahr W, Diehl A, Hermann D, Heinz A, Mann K.** Monitoring the effects of chronic alcohol consumption and abstinence on brain metabolism: a longitudinal 1H MRSI study. *Biol Psychiatry* 58: 974–980, 2005.
131. **Engblom D, Bilbao A, Sanchis-Segura C, Dahan L, Perreau-Lenz S, Balland B, Parkitna JR, Luján R, Halbout B, Mameli M, Parlato R, Sprengel R, Lüscher C, Schütz G, Spanagel R.** Glutamate receptors on dopamine neurons control the persistence of cocaine seeking. *Neuron* 59: 497–508, 2008.
132. **Engel SR, Lyons CR, Allan AM.** 5-HT_3 receptor over-expression decreases ethanol self administration in transgenic mice. *Psychopharmacology* 140: 243–248, 1998.
133. **Eravci M, Großpietsch T, Pinna G, Schulz O, Kley S, Bachmann M, Wolffgramm J, Götz E, Heyne A, Meinhold H, Baumgartner A.** Dopamine receptor gene expression in an animal model of behavioral dependence on ethanol. *Mol Brain Res* 50: 221–229, 1997.
134. **Ericson M, Blomqvist O, Engel JA, Söderpalm B.** Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur J Pharmacol* 358: 189–196, 1998.
135. **Ericson M, Molander A, Löf E, Engel JA, Söderpalm B.** Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *Eur J Pharmacol* 467: 85–93, 2003.
136. **Ericson M, Lof E, Stomberg R, Chau P, Soderpalm B.** Nicotinic acetylcholine receptors in the anterior, but not posterior, VTA mediate ethanol induced elevation of accumbal dopamine levels. *J Pharmacol Exp Ther* 326: 76–82, 2008.
137. **Eriksson K.** Genetic selection for voluntary alcohol consumption in the albino rat. *Science* 159: 739–741, 1968.
138. **Everitt BJ, Robbins TW.** Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* 8: 1481–1489, 2005.
139. **Fadel J, Deutch AY.** Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* 111: 379–387, 2002.
140. **Fahlke C, Engel JA, Eriksson CJ, Hard E, Soderpalm B.** Involvement of corticosterone in the modulation of ethanol consumption in the rat. *Alcohol* 11: 195–202, 2004.
141. **Fahlke C, Eriksson CJ.** Effect of adrenalectomy and exposure to corticosterone on alcohol intake in alcohol-preferring and alcohol-avoiding rat lines. *Alcohol Alcohol* 35: 139–144, 2000.
142. **Fahlke C, Hard E, Hansen S.** Facilitation of ethanol consumption by intracerebroventricular infusions of corticosterone. *Psychopharmacology* 127: 133–139, 1996.

143. **Fattore L, Cossu G, Spano MS, Deiana S, Fadda P, Scherma M, Fratta W.** Cannabinoids and reward: interactions with the opioid system. *Crit Rev Neurobiol* 16: 147–158, 2004.
144. **Flatscher-Bader T, van der BM, Hwang JW, Gochee PA, Matsumoto I, Niwa S, Wilce PA.** Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. *J Neurochem* 93: 359–370, 2005.
145. **Finley JC, Lindstrom P, Petrusz P.** Immunocytochemical localization of β-endorphin-containing neurons in the rat brain. *Neuroendocrinology* 33: 28–42, 1981.
146. **Freund TF, Katona I, Piomelli D.** Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 83: 1017–1066, 2003.
147. **Füllgrabe M, Vengeliene V, Spanagel R.** Influence of age at drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats. *Pharmacol Biochem Behav* 86: 320–327, 2007.
148. **Gass JT, Olive MF.** Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol* 75: 218–265, 2008.
149. **Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li TK.** Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* 11: 557–564, 1994.
150. **Gebicke-Haerter PJ.** Expression profiling methods used in drug abuse research. *Addict Biol* 10: 37–46, 2005.
151. **Gebicke-Haerter PJ, Sommer WH.** DNA microarrays and expression profiling in drug abuse research. *Addict Biol* 10: 1–3, 2005.
152. **Gelernter J, Gueorguieva R, Kranzler HR, Zhang H, Cramer J, Rosenheck R, Krystal JH; Cooperative Study #425 Study Group VA.** Opioid receptor gene (OPRM1, OPRK1, and OPRD1) variants and response to naltrexone treatment for alcohol dependence: results from the VA Cooperative Study. *Alcohol Clin Exp Res* 31: 555–563, 2007.
153. **George MS, Anton RF, Bloomer C, Teneback C, Drobes DJ, Lorberbaum JP, Nahas Z, Vincent DJ.** Activation of prefrontal cortex and anterior thalamus in alcoholic subjects on exposure to alcohol-specific cues. *Arch Gen Psychiatry* 58: 345–352, 2001.
154. **George DT, Gilman J, Hersh J, Thorsell A, Herion D, Geyer C, Peng X, Kielbasa W, Rawlings R, Brandt JE, Gehlert DR, Tauscher JT, Hunt SP, Hommer D, Heilig M.** Neurokinin 1 receptor antagonism as a possible therapy for alcoholism. *Science* 319: 1536–1539, 2008.
155. **Georgiadis JR, Kortekaas R, Kuipers R, Nieuwenburg A, Pruim J, Reinders AA, Holstege G.** Regional cerebral blood flow changes associated with clitorally induced orgasm in healthy women. *Eur J Neurosci* 24: 3305–3316, 2006.
156. **Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM.** It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* 26: 184–192, 2003.
157. **Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G.** Low doses of ethanol activate dopaminergic neurons of the ventral tegmental area. *Brain Res* 348: 201–203, 1985.
158. **Gessa GL, Serra S, Vacca G, Carai MA, Colombo G.** Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. *Alcohol Alcohol* 40: 46–53, 2005.
159. **Gilman JM, Hommer DW.** Modulation of brain response to emotional images by alcohol cues in alcohol-dependent patients. *Addict Biol* 2008.
160. **Gilpin NW, Stewart RB, Murphy JM, Li TK, Badia-Elder NE.** Neuropeptide Y reduces oral ethanol intake in alcohol-preferring (P) rats following a period of imposed ethanol abstinence. *Alcohol Clin Exp Res* 27: 787–794, 2003.
161. **Giorgetti M, Hotsenpiller G, Ward P, Teppen T, Wolf ME.** Amphetamine-induced plasticity of AMPA receptors in the ventral tegmental area: effects on extracellular levels of dopamine and glutamate in freely moving rats. *J Neurosci* 21: 6362–6369, 2001.
162. **Goeders NE, Lane JD, Smith JE.** Self-administration of methionine enkephalin into the nucleus accumbens. *Pharmacol Biochem Behav* 20: 451–455, 1984.
163. **Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, Watsky EJ, Gong J, Williams KE, Reeves KR; Varenicline Phase 3 Study Group.** Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs. sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA* 296: 47–55, 2006.
164. **Gonzales RA, Weiss F.** Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. *J Neurosci* 18: 10663–10671, 1998.
165. **Grant KA.** Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. *Behav Pharmacol* 5: 383–404, 1994.
166. **Grant KA, Colombo G.** Discriminative stimulus effects of ethanol: effect of training dose on the substitution of *N*-methyl-D-aspartate antagonists. *J Pharmacol Exp Ther* 264: 1241–1247, 1993.
167. **Grant BF, Dawson DA.** Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse* 9: 103–110, 1997.
168. **Greengard P, Allen PB, Nairn AC.** Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23: 435–447, 1999.
169. **Grüsser SM, Wrase J, Klein S, Hermann D, Smolka MN, Ruf M, Weber-Fahr W, Flor H, Mann K, Braus DF, Heinz A.** Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology* 175: 296–302, 2004.
170. **Hansson AC, Bermudez-Silva FJ, Marinen H, Hyytia P, Sanchez-Vera I, Rimondini R, Rodriguez de Fonseca F, Kunos G, Sommer WH, Heilig M.** Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology* 32: 117–126, 2007.
171. **Hansson AC, Cippitelli A, Sommer W, Ciccocioppo R, Heilig M.** Region-specific down regulation of *Crhr1* gene expression in alcohol preferring msP rats following ad lib access to alcohol. *Addict Biol* 12: 30–34, 2007.
172. **Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Terasmaa A, Massi M, Heilig M, Ciccocioppo R.** Variation at the rat *Crhr1* locus and sensitivity to relapse into alcohol seeking induced by environmental stress. *Proc Natl Acad Sci USA* 103: 15236–15241, 2006.
173. **Hansson AC, Rimondini R, Neznanova O, Sommer WH, Heilig M.** Neuroplasticity in brain reward circuitry following a history of ethanol dependence. *Eur J Neurosci* 27: 1912–1922, 2008.
174. **Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR.** Serotonin transporter genetic variation and the response of the human amygdala. *Science* 297: 400–403, 2002.
175. **Harper C, Dixon G, Sheedy D, Garrick T.** Neuropathological alterations in alcoholic brains. Studies arising from the New South Wales Tissue Resource Centre. *Prog Neuropsychopharmacol Biol Psychiatry* 27: 951–961, 2003.
176. **Harris GC, Aston-Jones G.** Arousal and reward: a dichotomy in orexin function. *Trends Neurosci* 29: 571–577, 2006.
177. **Harris RA, McQuilkin SJ, Paylor R, Abeliovich A, Tonegawa S, Wehner JM.** Mutant mice lacking the gamma isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of gamma-aminobutyrate type A receptors. *Proc Natl Acad Sci USA* 92: 3658–3662, 1995.
178. **Harris RA.** Ethanol actions on multiple ion channels: which are important? *Alcohol Clin Exp Res* 23: 1563–1570, 1999.
179. **Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC.** Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 64: 193–200, 2007.
180. **Heilig M, Egli M.** Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. *Pharmacol Ther* 111: 855–876, 2006.
181. **Heilig M, Koob GF.** A key role for corticotropin-releasing factor in alcohol dependence. *Trends Neurosci* 30: 399–406, 2007.
182. **Heilig M, Koob GF, Ekman R, Britton KT.** Corticotropin-releasing factor and neuropeptide Y: role in emotional integration. *Trends Neurosci* 17: 80–85, 1994.
183. **Heimer L, Alheid GF.** Piecing together the puzzle of basal forebrain anatomy. *Adv Exp Med Biol* 295: 1–42, 1991.

184. **Heinz A, Dufeu P, Kuhn S, Dettling M, Graef KJ, Kuerten I, Rommelspacher H, Schmidt LG.** Psychopathological and behavioral correlates of dopaminergic sensitivity in alcohol-dependent patients. *Arch Gen Psychiatry* 53: 1123–1128, 1996.
185. **Heinz A, Reimold M, Wrase J, Hermann D, Croissant B, Mundle G, Dohmen BM, Braus DF, Schumann G, Machulla HJ, Bares R, Mann K.** Correlation of stable elevations in striatal mu-opioid receptor availability in detoxified alcoholic patients with alcohol craving: a positron emission tomography study using carbon 11-labeled carfentanil. *Arch Gen Psychiatry* 62: 57–64, 2005.
186. **Heinz A, Siessmeier T, Wrase J, Buchholz HG, Gründer G, Kumakura Y, Cumming P, Schreckenberger M, Smolka MN, Rösch F, Mann K, Bartenstein P.** Correlation of alcohol craving with striatal dopamine synthesis capacity and D2/3 receptor availability: a combined [^{18}F]DOPA and [^{18}F]DMFP PET study in detoxified alcoholic patients. *Am J Psychiatry* 162: 1515–1520, 2005.
187. **Hendriks HF, van Tol A.** Alcohol. *Handb Exp Pharmacol* 170: 3393–3461, 2005.
188. **Henniger MS, Spanagel R, Wigger A, Landgraf R, Hölter SM.** Alcohol self-administration in two rat lines selectively bred for extremes in anxiety-related behavior. *Neuropsychopharmacology* 26: 729–736, 2002.
189. **Herz A.** Endogenous opioid systems and alcohol addiction. *Psychopharmacology* 129: 99–111, 1997.
190. **Hesselbrock MN, Meyer RE, Keener JJ.** Psychopathology in hospitalized alcoholics. *Arch Gen Psychiatry* 42: 1050–1055, 1985.
191. **Hill SY, De Bellis MD, Keshavan MS, Lowers L, Shen S, Hall J, Pitts T.** Right amygdala volume in adolescent and young adult offspring from families at high risk for developing alcoholism. *Biol Psychiatry* 49: 894–905, 2001.
192. **Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF, Wang D, Sanchez-Perez AM, Messing RO.** Supersensitivity to allosteric GABA(A) receptor modulators and alcohol in mice lacking PKCepsilon. *Nat Neurosci* 2: 997–1002, 1999.
193. **Hodge CW, Grant KA, Becker HC, Besheer J, Crissman AM, Platt DM, Shannon EE, Shelton KL.** Understanding how the brain perceives alcohol: neurobiological basis of ethanol discrimination. *Alcohol Clin Exp Res* 30: 203–213, 2006.
194. **Hodge CW, Samson HH, Chappelle AM.** Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcohol Clin Exp Res:* 21, 1083–1091, 1997.
195. **Hölter SM, Danysz W, Spanagel R.** Evidence for alcohol anticraving properties of memantine. *Eur J Pharmacol* 314: R1–2, 1996.
196. **Hölter SM, Engelmann M, Kirschke C, Liebsch G, Landgraf R, Spanagel R.** Long-term ethanol self-administration with repeated ethanol deprivation episodes changes ethanol drinking pattern and increases anxiety-related behaviour during ethanol deprivation in rats. *Behav Pharmacol* 9: 41–48, 1998.
197. **Hölter SM, Spanagel R.** Effects of opiate antagonist treatment on the alcohol deprivation effect in long-term ethanol-experienced rats. *Psychopharmacology* 145: 360–369, 1999.
198. **Hölter SM, Danysz W, Spanagel R.** The non-competitive NMDA receptor antagonist MRZ 2/579 suppresses the alcohol deprivation effect in long-term alcohol drinking rats and substitutes the alcohol cue in a discrimination task. *J Pharmacol Exp Ther* 292: 545–552, 2000.
199. **Hölter SM, Henniger MS, Lipkowski AW, Spanagel R.** Kappa-opioid receptors and relapse-like drinking in long-term ethanol experienced rats. *Psychopharmacology* 153: 93–102, 2002.
200. **Hofmann F, Feil R, Kleppisch T, Schlossmann J.** Function of cGMP-dependent protein kinases as revealed by gene deletion. *Physiol Rev* 86: 1–23, 2006.
201. **Hoffman P, Tabakoff B.** Gene expression in animals with different acute responses to ethanol. *Addict Biol* 10: 63–69, 2005.
202. **Holstege G, Georgiadis JR, Paans AM, Meiners LC, van der Graaf FH, Reinders AA.** Brain activation during human male ejaculation. *J Neurosci* 23: 9185–9193, 2003.
203. **Honse Y, Ren H, Lipsky RH, Peoples RW.** Sites in the fourth membrane-associated domain regulate alcohol sensitivity of the NMDA receptor. *Neuropharmacology* 46: 647–654, 2004.
204. **Howell LL, Wilcox KM.** Functional imaging and neurochemical correlates of stimulant self-administration in primates. *Psychopharmacology* 163: 352–361, 2002.
205. **Howland JG, Taepavarapruk P, Phillips AG.** Glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens by basolateral, but not central, nucleus of the amygdala in rats. *J Neurosci* 22: 1137–1145, 2002.
206. **Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR.** Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258: 577–580, 1975.
207. **Hultberg B, Berglund M, Andersson A, Frank A.** Elevated plasma homocysteine in alcoholics. *Alcohol Clin Exp Res* 17: 687–689, 1993.
208. **Hummler E, Cole TJ, Blendy JA, Ganss R, Aguzzi A, Schmid W, Beermann F, Schutz G.** Targeted mutation of the CREB gene: compensation within the CREB/ATF family of transcription factors. *Proc Natl Acad Sci USA* 91: 5647–5651, 1994.
209. **Hundt W, Hölter SM, Spanagel R.** Discriminative stimulus effects of glutamate release inhibitors in rats trained to discriminate ethanol. *Pharmacol Biochem Behav* 59: 691–695, 1998.
210. **Hungund BL, Basavarajappa BS.** Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *J Neurosci Res* 60: 122–128, 2000.
211. **Hyytiä P, Kiianmaa K.** Suppression of ethanol responding by centrally administered CTOP and naltrindole in AA and Wistar rats. *Alcohol Clin Exp Res* 25: 25–33, 2001.
212. **Ikemoto S, McBride WJ, Murphy JM, Lumeng L, Li TK.** 6-OHDA lesions of the nucleus accumbens disrupt the acquisition but not the maintenance of ethanol consumption in the alcohol-preferring P line of rats. *Alcohol Clin Exp Res* 21: 1042–1046, 1997.
213. **Imperato A, Di Chiara G.** Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther* 239: 219–239, 1986.
214. **Jackson A, Stephens DN, Duka T.** A low dose alcohol drug discrimination in social drinkers: relationship with subjective effects. *Psychopharmacology* 157: 411–420, 2001.
215. **Jaffe JH, Babor TF, Fischbein TH.** Alcoholics, aggression and antisocial personality. *J Stud Alcohol* 49: 211–218, 1988.
216. **Jamensky NT, Gianoulakis C.** Content of dynorphins and κ-opioid receptors in distinct brain regions of C57BL/6 and DBA/2 mice. *Alcohol Clin Exp Res* 21: 1455–1464, 1997.
217. **Janak PH, Chang JY, Woodward DJ.** Neuronal spike activity in the nucleus accumbens of behaving rats during ethanol self-administration. *Brain Res* 817: 172–184, 1999.
218. **Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA.** Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict Biol* 11: 45–54, 2006.
219. **Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA.** Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol* 12: 6–16, 2007.
220. **Johnson BA, Ait-Daoud N, Bowden CL, DiClemente CC, Roache JD, Lawson K, Javors MA, Ma JZ.** Oral topiramate for treatment of alcohol dependence: a randomised controlled trial. *Lancet* 361: 1677–1685, 2003.
221. **Johnson BA, Roache JD, Javors MA, DiClemente CC, Cloninger CR, Prihoda TJ, Bordnick PS, Ait-Daoud N, Hensler J.** Ondansetron for reduction of drinking among biologically predisposed alcoholic patients: a randomized controlled trial. *JAMA* 284: 963–971, 2000.
222. **Johnson BA, Rosenthal N, Capece JA, Wiegand F, Mao L, Beyers K, McKay A, Ait-Daoud N, Anton RF, Ciraulo DA, Kranzler HR, Mann K, O'Malley SS, Swift RM; Topiramate for Alcoholism Advisory Board; Topiramate for Alcoholism Study Group.** Topiramate for treating alcohol dependence: a randomized controlled trial. *JAMA* 298: 1641–1651, 2007.
223. **Jones S, Kornblum JL, Kauer JA.** Amphetamine blocks long-term synaptic depression in the ventral tegmental area. *J Neurosci* 20: 5575–5580, 2000.
224. **Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR; Varenicline Phase 3 Study Group.** Efficacy of varenicline, an alpha4beta2

nicotinic acetylcholine receptor partial agonist, vs. placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA* 296: 56–63, 2006.
225. **June HL, Torres L, Cason CR, Hwang BH, Braun MR, Murphy JM.** The novel benzodiazepine inverse agonist RO19–4603 antagonizes ethanol motivated behaviors: neuropharmacological studies. *Brain Res* 784: 256–275, 1998.
226. **June Sr HL, Foster KL, Eiler 2nd WJ, Goergen J, Cook JB, Johnson N, Mensah-Zoe B, Simmons JO, June HL Jr, Yin W, Cook JM, Homanics GE.** Dopamine and benzodiazepine-dependent mechanisms regulate the EtOH-enhanced locomotor stimulation in the $GABA_A$ alpha1 subunit null mutant mice. *Neuropsychopharmacology* 32: 137–152, 2007.
227. **Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U.** General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J* 17: 250–262, 2003.
228. **Kalivas PW.** Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Rev* 18: 75–113, 1993.
229. **Kalivas PW, Stewart J.** Dopamine transmission in the initation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev* 16: 223–244, 1991.
230. **Kampe KK, Frith CD, Dolan RJ, Frith U.** Reward value of attractiveness and gaze. *Nature* 413: 589, 2001.
231. **Katner SN, Kerr TM, Weiss F.** Ethanol anticipation enhances dopamine efflux in the nucleus accumbens of alcohol-preferring (P) but not Wistar rats. *Behav Pharmacol* 7: 669–674, 1996.
232. **Katner SN, Magalong JG, Weiss F.** Reinstatement of alcohol-seeking behavior by drug-associated discriminative stimuli after prolonged extinction in the rat. *Neuropsychopharmacology* 20: 471–479, 1999.
233. **Katner SN, Weiss F.** Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and -nonpreferring rats: a quantitative microdialysis study. *Alcohol Clin Exp Res* 25: 198–205, 2001.
234. **Kauer JA, Malenka RC.** Synaptic plasticity and addiction. *Nat Rev Neurosci* 8: 844–858, 2007.
235. **Kelaï S, Aïssi F, Lesch KP, Cohen-Salmon C, Hamon M, Lanfumey L.** Alcohol intake after serotonin transporter inactivation in mice. *Alcohol Alcohol* 38: 386–389, 2003.
236. **Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, Williams RW, Miles MF.** Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci* 25: 2255–2266, 2005.
237. **Kerns RT, Miles MF.** Microarray analysis of ethanol-induced changes in gene expression. *Methods Mol Biol* 447: 395–410, 2008.
238. **Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, Kendler KS.** Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 51: 8–19, 1994.
239. **Kiefer F, Spanagel R.** Measuring alcohol consumption in man. It's time for a change. *Addiction* 101: 1214–1218, 2006.
240. **Kiefer F, Wiedemann K.** Neuroendocrine pathways of addictive behaviour. *Addict Biol* 9: 205–212, 2004.
241. **Kiianmaa K, Andersson K, Fuxe K.** On the role of ascending dopamine systems in the control of voluntary ethanol intake and ethanol intoxication. *Pharmacol Biochem Behav* 10: 603–618, 1979.
242. **Kiianmaa K, Nurmi M, Nykanen I, Sinclair JD.** Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferrring AA and alcohol-avoiding ANA rats. *Pharmacol Biochem Behav* 52: 29–34, 1995.
243. **Kiianmaa K, Stenius K, Sinclair JD.** Determinants of alcohol preference in the AA and ANA rat lines selected for differential ethanol intake. *Alcohol Alcohol* 26: 115–120, 1991.
244. **Kim MS, Repp A, Smith DP.** LUSH odorant-binding protein mediates chemosensory responses to alcohols in *Drosophila melanogaster*. *Genetics* 150: 711–721, 1998.
245. **Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K, Ijiri I, Harbuz MS.** Acute ethanol decreases NPY mRNA but not POMC mRNA in the arcuate nucleus. *Neuroreport* 11: 3517–3519, 2000.
246. **Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T.** Ethanol opens G-protein activated inwardly rectifying K^+ channels. *Nat Neurosci* 2: 1091–1097, 1999.
247. **Kohl RR, Katner JS, Chernet E, McBride WJ.** Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology* 139: 79–85, 1998.
248. **Koob GF.** Neuroadaptive mechanisms of addiction: studies on the extended amygdale. *Eur Neuropsychopharmacol* 13: 442–452, 2003.
249. **Koob GF.** A role for brain stress systems in addiction. *Neuron* 59: 11–34, 2008.
250. **Koob GF, Le Moal M.** Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24: 97–129, 2001.
251. **Kostowski W, Bienkowski P.** Discriminative stimulus effects of ethanol: neuropharmacological characterization. *Alcohol* 17: 63–80, 1999.
252. **Kovacs KM, Szakall I, O'Brien D, Wang R, Vinod KY, Saito M, Simonin F, Kieffer BL, Vadasz C.** Decreased oral self-administration of alcohol in kappa-opioid receptor knockout mice. *Alcohol Clin Exp Res* 29: 730–738, 2005.
253. **Kranzler HR, Pierucci-Lagha A, Feinn R, Hernandez-Avila C.** Effects of ondansetron in early- versus late-onset alcoholics: a prospective, open-label study. *Alcohol Clin Exp Res* 27: 1150–1155, 2003.
254. **Kruse SW, Zhao R, Smith DP, Jones DN.** Structure of a specific alcohol-binding site defined by the odorant binding protein LUSH from *Drosophila melanogaster*. *Nat Struct Biol* 10: 694–700, 2003.
255. **Krystal JH, Petrakis IL, Webb E, Cooney NL, Karper LP, Namanworth S, Stetson P, Trevisan LA, Charney DS.** Dose-related ethanol-like effects of the NMDA antagonist, ketamine, in recently detoxified alcoholics. *Arch Gen Psychiatry* 55: 354–360, 1998.
256. **Kuehner C.** Gender differences in unipolar depression: an update of epidemiological findings and possible explanations. *Acta Psychiatr Scand* 108: 163–174, 2003.
257. **Kuzmin A, Kreek MJ, Bakalkin G, Liljequist S.** The nociceptin/orphanin FQ receptor agonist Ro 64–6198 reduces alcohol self-administration and prevents relapse-like alcohol drinking. *Neuropsychopharmacology* 32: 902–910, 2007.
258. **Lancaster FE, Brown TD, Coker KL, Elliott JA, Wren SB.** Sex differences in alcohol preference and drinking patterns emerge during the early postpubertal period. *Alcohol Clin Exp Res* 16: 1043–1049, 1996.
259. **Landgraf R, Wigger A.** High vs. low anxiety-related behavior rats: an animal model of extremes in trait anxiety. *Behav Genet* 32: 301–314, 2002.
260. **Larsson A, Jerlhag E, Svensson L, Söderpalm B, Engel JA.** Is an alpha-conotoxin MII-sensitive mechanism involved in the neurochemical, stimulatory, and rewarding effects of ethanol? *Alcohol* 34: 239–250, 2004.
261. **Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K.** GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci USA* 105: 2699–2704, 2008.
262. **Lawrence AJ, Cowen MS, Yang HJ, Chen F, Oldfield B.** The orexin system regulates alcohol-seeking in rats. *Br J Pharmacol* 148: 752–759, 2006.
263. **Lê AD, Funk R.** Serotonergic compounds: Preclinical data. In: *Drugs for Relapse Prevention of Alcoholism*, edited by Spanagel R, Mann K. Basel: Birkhäuser, 2005, p. 95–106.
264. **Lê AD, Funk D, Harding S, Juzytsch W, Fletcher PJ, Shaham Y.** Effects of dexfenfluramine and $5\text{-}HT_3$ receptor antagonists on stress-induced reinstatement of alcohol seeking in rats. *Psychopharmacology* 186: 82–92, 2006.
265. **Lê AD, Poulos CX, Harding S, Watchus J, Juzytsch W, Shaham Y.** Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injections of alcohol and exposure to stress. *Neuropsychopharmacology* 21: 435–444, 1999.
266. **Lê AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y.** Reinstatement of alcohol-seeking by priming injections of alco-

hol and exposure to stress in rats. *Psychopharmacology* 135: 169–174, 1998.
267. **Lee H, Holburn GH, Price RR.** Proton MR spectroscopic studies of chronic alcohol exposure on the rat brain. *J Magn Reson Imaging* 18: 147–151, 2003.
268. **Lévesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P.** Identification, characterization, and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2aminotetralin. *Proc Natl Acad Sci USA* 89: 8155–8159, 1992.
269. **Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA.** G protein-coupled inwardly rectifying potassium channels are targets of alcohol action. *Nat Neurosci* 2: 1084–1090, 1999.
270. **Li TK, Hewitt BG, Grant BF.** The alcohol dependence syndrome, 30 years later: a commentary. The 2006 H David Archibald lecture. *Addiction* 102: 1522–1530, 2007.
271. **Liang T, Spence J, Liu L, Strother WN, Chang HW, Ellison JA, Lumeng L, Li TK, Foroud T, Carr LG.** α-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. *Proc Natl Acad Sci USA* 100: 4690–4695, 2003.
272. **Little HJ.** Behavioral mechanisms underlying the link between smoking and drinking. *Alcohol Res Health* 24: 215–224, 2004.
273. **Liu J, Lewohl JM, Harris RA, Iyer VR, Dodd PR, Randall PK, Mayfield RD.** Patterns of gene expression in the frontal cortex discriminate alcoholic from nonalcoholic individuals. *Neuropsychopharmacology* 31: 1574–1582, 2006.
274. **Liu QS, Pu L, Poo MM.** Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature* 437: 1027–1031, 2005.
275. **Liu W, Thielen RJ, Rodd ZA, McBride WJ.** Activation of serotonin-3 receptors increases dopamine release within the ventral tegmental area of Wistar and alcohol-preferring (P) rats. *Alcohol* 40: 167–176, 2006.
276. **Liu X, Weiss F.** Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. *J Neurosci* 22: 7856–7861, 2002.
277. **Liu X, Weiss F.** Reversal of ethanol-seeking behavior by D1 and D2 antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. *J Pharmacol Exp Ther* 300: 882–889, 2002.
278. **Löf E, Ericson M, Stomberg R, Söderpalm B.** Characterization of ethanol-induced dopamine elevation in the rat nucleus accumbens. *Eur J Pharmacol* 555: 148–155, 2007.
279. **Löf E, Olausson P, deBejczy A, Stomberg R, McIntosh JM, Taylor JR, Söderpalm B.** Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues. *Psychopharmacology* 195: 333–343, 2007.
280. **Lonze BE, Ginty DD.** Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35: 605–623, 2002.
281. **López-Moreno JA, González-Cuevas G, Moreno G, Navarro M.** The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict Biol* 13: 160–187, 2008.
282. **Lovinger DM, White G.** Ethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. *Mol Pharmacol* 40: 263–270, 1991.
283. **Lovinger DM, White G, Weight FF.** Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243: 1721–1724, 1989.
284. **Lovinger DM, Zhou Q.** Alcohol effects on the 5-HT$_3$ ligand-gated ion channel. *Toxicol Lett* 100–101: 239–246, 1998.
285. **Ludwig AM, Wikler A, Stark LH.** The first drink: psychobiological aspects of craving. *Arch Gen Psychiatry* 30: 539–471, 1974.
286. **Luscher C, Ungless MA.** The mechanistic classification of addictive drugs. *PLoS Med* 3: e437, 2006.
287. **Lyness WH, Smith FL.** Influence of dopaminergic and serotonergic neurons on intravenous ethanol self-administration in the rat. *Pharmacol Biochem Behav* 42: 187–192, 1992.
288. **Macenski MJ, Shelton KL.** Self-administered ethanol as a discriminative stimulus in rats. *Drug Alcohol Depend* 64: 243–247, 2001.
289. **Machu TK, Harris RA.** Alcohols and anesthetics enhance the function of 5-hydroxytryptamine$_3$ receptors expressed in *Xenopus laevis* oocytes. *J Pharmacol Exp Ther* 271: 898–905, 1994.
290. **Madeira MD, Paula-Barbosa MM.** Effects of alcohol on the synthesis and expression of hypothalamic peptides. *Brain Res Bull* 48: 3–22, 1999.
291. **Maldonado F, Valverde O, Berrendero F.** Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* 29: 225–232, 2006.
292. **Maldve RE, Zhang TA, Ferrani-Kile K, Schreiber SS, Lippmann MJ, Snyder GL, Fienberg AA, Leslie SW, Gonzales RA, Morrisett RA.** DARPP-32 and regulation of the ethanol sensitivity of NMDA receptors in the nucleus accumbens. *Nat Neurosci* 5: 641–648, 2002.
293. **Mameli M, Balland B, Lujan R, Luscher C.** Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* 317: 530–533, 2007.
294. **Mann K, Ackermann K, Diehl A, Ebert D, Mundle G, Nakovics H, Reker T, Richter G, Schmidt LG, Driessen M, Rettig K, Opitz K, Croissant B.** Galantamine: a cholinergic patch in the treatment of alcoholism: a randomized, placebo-controlled trial. *Psychopharmacology* 184: 115–121, 2006.
295. **Mann K, Kiefer F, Spanagel R, Littleton J.** Acamprosate: new findings and future research directions. *Alcohol Clin Exp Res.* 32: 1105–1110, 2008.
296. **Mann K, Lehert P, Morgan MY.** The efficacy of acamprosate in the maintenance of abstinence in alcohol-dependent individuals: results of a meta-analysis. *Alcohol Clin Exp Res* 28: 51–63, 2004.
297. **Marinelli PW, Funk D, Juzytsch W, Harding S, Rice KC, Shaham Y, Lê AD.** The CRF1 receptor antagonist antalarmin attenuates yohimbine-induced increases in operant alcohol self-administration and reinstatement of alcohol seeking in rats. *Psychopharmacology* 195: 345–355, 2007.
298. **Marinelli PW, Quirion R, Gianoulakis C.** A microdialysis profile of beta-endorphin and catecholamines in the rat nucleus accumbens following alcohol administration. *Psychopharmacology* 169: 60–67, 2003.
299. **Marinelli PW, Quirion R, Gianoulakis C.** An in vivo profile of beta-endorphin release in the arcuate nucleus and nucleus accumbens following exposure to stress or alcohol. *Neuroscience* 127: 777–784, 2004.
300. **Markou A, Kosten TR, Koob GF.** Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology* 18: 135–174, 1998.
301. **Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A, Kegeles L, Talbot P, Evans S, Krystal J, Laruelle M, Abi-Dargham A.** Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biol Psychiatry* 58: 779–786, 2005.
302. **Mattson MP, Shea TB.** Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci* 26: 137–146, 2003.
303. **May T, Wolf U, Wolffgramm J.** Striatal dopamine receptors and adenylyl cyclase activity in a rat model of alcohol addiction: effects of ethanol and lisuride treatment. *J Pharmacol Exp Ther* 275: 1195–1203, 1995.
304. **Mayer D, Zahr NM, Sullivan EV, Pfefferbaum A.** In vivo metabolite differences between the basal ganglia and cerebellum of the rat brain detected with proton MRS at 3T. *Psychiatry Res* 154: 267–273, 2007.
305. **Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA.** Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. *J Neurochem* 81: 802–813, 2002.
306. **Mayfield RD, Harris RA, Schuckit MA.** Genetic factors influencing alcohol dependence. *Br J Pharmacol* 154: 275–287, 2008.

307. **McBride WJ, Li TK.** Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol* 12: 339–369, 1998.
308. **McBride WJ, Lovinger DM, Machu T, Thielen RJ, Rodd ZA, Murphy JM, Roache JD, Johnson BA.** Serotonin-3 receptors in the actions of alcohol, alcohol reinforcement, and alcoholism. *Alcohol Clin Exp Res* 28: 257–267, 2004.
309. **McCaul ME, Wand GS, Eissenberg T, Rohde CA, Cheskin LJ.** Naltrexone alters subjective and psychomotor responses to alcohol in heavy drinking subjects. *Neuropsychopharmacology* 22: 480–492, 2000.
310. **McEwen BS.** Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 87: 873–904, 2007.
311. **McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Luong K, Kharazia V, Janak PH, Ron D.** RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. *J Neurosci* 24: 10542–10552, 2004.
312. **Melis M, Camarini R, Ungless MA, Bonci A.** Long-lasting potentiation of GABAergic synapses in dopamine neurons after a single in vivo ethanol exposure. *J Neurosci* 22: 2074–2082, 2002.
313. **Melis M, Spiga S, Diana M.** The dopamine hypothesis of drug addiction: hypodopaminergic state. *Int Rev Neurobiol* 63: 101–154, 2005.
314. **Merikangas KR, Risch NJ, Weissman MM.** Comorbidity and co-transmission of alcoholism, anxiety and depression. *Psychol Med* 24: 69–80, 1994.
315. **Meyer-Lindenberg A, Weinberger DR.** Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 7: 818–827, 2005.
316. **Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE.** GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res* 25: 1708–1718, 2001.
317. **Mihic SJ.** Acute effects of ethanol on $GABA_A$ and glycine receptor function. *Neurochem Int* 35: 115–123, 1999.
318. **Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL.** Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* 389: 385–389, 1997.
319. **Miller G.** Tackling alcoholism with drugs. *Science* 320: 168–170, 2008.
320. **Milliken CS, Auchterlonie JL, Hoge CW.** Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. *JAMA* 298: 2141–2148, 2007.
321. **Misra K, Pandey SC.** The decreased cyclic-AMP dependent-protein kinase A function in the nucleus accumbens: a role in alcohol drinking but not in anxiety-like behaviors in rats. *Neuropsychopharmacology* 31: 1406–1419, 2006.
322. **Misra K, Roy A, Pandey SC.** Effects of voluntary ethanol intake on the expression of Ca^{2+}/calmodulin-dependent protein kinase IV and on CREB expression and phosphorylation in the rat nucleus accumbens. *Neuroreport* 12: 4133–4137, 2001.
323. **Möller C, Wiklund L, Sommer W, Thorsell A, Heilig M.** Decreased experimental anxiety and voluntary ethanol consumption in rats following central but not basolateral amygdala lesions. *Brain Res* 760: 94–101, 1997.
324. **Moghaddam B, Bolinao ML.** Biphasic effect of ethanol on extracellular accumulation of glutamate in the hippocampus and the nucleus accumbens. *Neurosci Lett* 178: 99–102, 1994.
325. **Molander A, Lidö HH, Löf E, Ericson M, Söderpalm B.** The glycine reuptake inhibitor Org 25935 decreases ethanol intake and preference in male Wistar rats. *Alcohol Alcohol* 42: 11–18, 2007.
326. **Molander A, Söderpalm B.** Glycine receptors regulate dopamine release in the rat nucleus accumbens. *Alcohol Clin Exp Res* 29: 17–26, 2005.
327. **Molander A, Söderpalm B.** Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. *Alcohol Clin Exp Res* 29: 27–37, 2005.
328. **Moolten M, Kornetsky C.** Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. *Alcohol* 7: 221–225, 1990.
329. **Moore MS, DeZazzo J, Luk AY, Tully T, Singh CM, Heberlein U.** Ethanol intoxication in *Drosophila*: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* 93: 997–1007, 1998.
330. **Mucha RF, Herz A.** Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology* 86: 274–280, 1985.
331. **Mueller HT, Meador-Woodruff JH.** NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. *Schizophr Res* 71: 361–370, 2004.
332. **Murray AM, Ryoo HL, Gurevich E, Joyce JN.** Localization of dopamine D_3 receptors to mesolimbic and D_2 receptors to mesostriatal regions of human forebrain. *Proc Natl Acad Sci USA* 91: 11271–11275, 1994.
333. **Mylecharane EJ.** Ventral tegmental area 5-HT receptors: mesolimbic dopamine release and behavioural studies. *Behav Brain Res* 73: 1–5, 1996.
334. **Myrick H, Anton RF, Li X, Henderson S, Drobes D, Voronin K, George MS.** Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. *Neuropsychopharmacology* 29: 393–402, 2004.
335. **Myrick H, Anton RF, Li X, Henderson S, Randall PK, Voronin K.** Effect of naltrexone and ondansetron on alcohol cue-induced activation of the ventral striatum in alcohol-dependent people. *Arch Gen Psychiatry* 65: 466–475, 2008.
336. **Narahashi T, Aistrup GL, Marszalec W, Nagata K.** Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. *Neurochem Int* 35: 131–141, 1999.
337. **Nelson RJ, Demas GE, Huang PL, Fishman MC, Dawson VL, Dawson TM, Snyder SH.** Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* 378: 383–386, 1995.
338. **Nesse RM, Berridge KC.** Psychoactive drug use in evolutionary perspective. *Science* 278: 63–66, 1997.
339. **Newton PM, Ron D.** Protein kinase C and alcohol addiction. *Pharmacol Res* 55: 570–577, 2007.
340. **Niccols A.** Fetal alcohol syndrome and the developing socio-emotional brain. *Brain Cogn* 65: 135–142, 2007.
341. **Nicolelis MA, Ribeiro S.** Multielectrode recordings: the next steps. *Curr Opin Neurobiol* 12: 602–606, 2002.
342. **Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM.** Blocking GABA(A) receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. *Psychopharmacology* 139: 108–116, 1998.
343. **Nurmi M, Ashizawa T, Sinclair JD, Kiianmaa K.** Effect of prior ethanol experience on dopamine overflow in accumbens of AA and ANA rats. *Eur J Pharmacol* 315: 277–283, 1996.
344. **Nurmi M, Sinclair JD, Kiianmaa K.** Dopamine release during ethanol drinking in AA rats. *Alcohol Clin Exp Res* 22: 1628–1633, 1998.
345. **Nutt D, King LA, Saulsbury W, Blakemore C.** Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet* 369: 1047–1053, 2007.
346. **Nylander I, Hyytiä P, Forsander O, Terenius L.** Differences between alcohol-preferring (AA) and alcohol-avoiding (ANA) rats in the prodynorphin and proenkephalin systems. *Alcohol Clin Exp Res* 8: 1272–1279, 1994.
347. **Olds J, Millner P.** Positive reinforcement produced by electrical stimulation of septal area and other regions of the rat brain. *J Comp Physiol Psychol* 47: 419–426, 1954.
348. **Olive MF, Koenig HN, Nannini MA, Hodge CW.** Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. *J Neurosci* 21: RC184, 2001.
349. **O'Malley SS, Jaffe AJ, Chang G, Schottenfeld RS, Meyer RE, Rounsaville B.** Naltrexone and coping skills therapy for alcohol dependence. A controlled study. *Arch Gen Psychiatry* 49: 881–887, 1992.
350. **Omelchenko N, Sesack SR.** Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. *Neuroscience* 146: 1259–1274, 2007.
351. **Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP.** A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in

alcohol-dependent patients. *Neuropsychopharmacology* 28: 1546–1552, 2003.
352. **Oslin DW, Berrettini WH, O'Brien CP.** Targeting treatments for alcohol dependence: the pharmacogenetics of naltrexone. *Addict Biol* 11: 397–403, 2006.
353. **Overstreet DH, Halikas JA, Seredenin SB, Kampov-Polevoy AB, Viglinskaya IV, Kashevskaya O, Badishtov BA, Knapp DJ, Mormede P, Kiianmaa K, Li TK, Rezvani AH.** Behavioral similarities and differences among alcohol-preferring and -nonpreferring rats: confirmation by factor analysis and extension to additional groups. *Alcohol Clin Exp Res* 21: 840–848, 1997.
354. **Overstreet DH, Rezvani AH, Cowen M, Chen F, Lawrence AJ.** Modulation of high alcohol drinking in the inbred Fawn-Hooded (FH/Wjd) rat strain: implications for treatment. *Addict Biol* 11: 356–373, 2006.
355. **Overstreet DH, Rezvani AH, Janowsky DS.** Genetic animal models of depression and ethanol preference provide support for cholinergic and serotonergic involvement in depression and alcoholism. *Biol Psychiatry* 31: 919–936, 1992.
356. **Pandey SC, Roy A, Zhang H, Xu T.** Partial deletion of the cAMP response element-binding protein gene promotes alcohol-drinking behaviors. *J Neurosci* 24: 5022–5030, 2004.
357. **Pandey SC, Ugale R, Zhang H, Tang L, Prakash A.** Brain chromatin remodeling: a novel mechanism of alcoholism. *J Neurosci* 28: 3729–3737, 2008.
358. **Pandey SC, Zhang H, Roy A, Xu T.** Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. *J Clin Invest* 115: 2762–2773, 2005.
359. **Parks MH, Dawant BM, Riddle WR.** Longitudinal brain metabolic characterization of chronic alcoholics with proton magnetic resonance spectroscopy. *Alcohol Clin Exp Res* 26: 1368–1380, 2002.
360. **Parsons CG, Danysz W, Quack G.** Memantine and the aminoalkyl. Cyclohexane MRZ 2/579 are moderate affinity uncompetitive NMDA receptor antagonists–in vitro characterisation. *Amino Acids* 19: 157–166, 2000.
361. **Parsons MP, Li S, Kirouac GJ.** Functional and anatomical connection between the paraventricular nucleus of the thalamus and dopamine fibers of the nucleus accumbens. *J Comp Neurol* 500: 1050–1063, 2007.
362. **Pawlak CR, Sanchis-Segura C, Soewarto D, Wagner S, Hrabé de Angelis M, Spanagel R.** A phenotype-driven ENU mutagenesis screen for the identification of dominant mutations involved in alcohol consumption. *Mamm Genome* 19: 77–84, 2008.
363. **Peoples RW, Li C, Weight FF.** Lipid vs. protein theories of alcohol action in the nervous system. *Annu Rev Pharmacol Toxicol* 36: 185–201, 1996.
364. **Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ.** A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J Neurosci* 22: 3090–3099, 2002.
365. **Perkonigg A, Pfister H, Hofler M, Frohlich C, Zimmermann P, Lieb R, Wittchen HU.** Substance use and substance use disorders in a community sample of adolescents and young adults: incidence, age effects and patterns of use. *Eur Addict Res* 12: 187–196, 2006.
366. **Perreau-Lenz S, Zghoul T, Spanagel R.** Clock genes running amok. Clock genes and their role in drug addiction and depression. *EMBO Rep* 8: S20–23, 2007.
367. **Pert CB, Snyder SH.** Opiate receptor: demonstration in nervous tissue. *Science* 179: 1011–1014, 1973.
368. **Petersen OH, Sutton R.** Ca^{2+} signaling and pancreatitis: effects of alcohol, bile and coffee. *Trends Pharmacol Sci* 27: 113–120, 2006.
369. **Pfeffer AO, Samson HH.** Haloperidol and apomorphine effects on ethanol reinforcement in free feeding rats. *Pharmacol Biochem Behav* 29: 343–350, 1988.
370. **Pfefferbaum A.** Alcoholism damages the brain, but does moderate alcohol use? *Lancet Neurol* 3: 143–144, 2004.
371. **Pfeiffer A, Brantl V, Herz A, Emrich HM.** Psychotomimesis mediated by κ opiate receptors. *Science* 233: 774–776, 1986.
372. **Pfeuffer J, Tkac I, Provencher SW, Gruetter R.** Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time ^{1}H NMR spectra of the rat brain. *J Magn Reson* 141: 104–120, 1999.
373. **Phillips TJ, Brown KJ, Burkhart-Kasch S, Wenger CD, Kelly MA, Rubinstein M, Grandy DK, Low MJ.** Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nat Neurosci* 1: 610–615, 1998.
374. **Phillips TJ, Wenger CD, Dorow JD.** Naltrexone effects on ethanol drinking acquisition and on established ethanol consumption in C57BL/6J mice. *Alcohol Clin Exp Res* 21: 691–702, 1997.
375. **Piazza PV, Le Moal M.** Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res Rev* 25: 359–372, 1997.
376. **Pihl RO, LeMarquand D.** Serotonin and aggression and the alcohol-aggression relationship. *Alcohol Alcohol* 33: 55–65, 1988.
377. **Pohorecky LA.** Interaction of ethanol and stress: research with experimental animals–an update. *Alcohol Alcohol* 25: 263–276, 1990.
378. **Pohorecky LA.** Stress and alcohol interaction: an update of human research. *Alcohol Clin Exp Res* 15: 438–459, 1991.
379. **Pontieri FE, Tanda G, Di Chiara G.** Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core"of the rat nucleus accumbens. *Proc Natl Acad Sci USA* 92: 12304–12308, 1995.
380. **Poon MM, Choi SH, Jamieson CA, Geschwind DH, Martin KC.** Identification of process-localized mRNAs from cultured rodent hippocampal neurons. *J Neurosci* 26: 13390–13399, 2006.
381. **Quertemont E, Eriksson CJ, Zimatkin SM, Pronko PS, Diana M, Pisano M, Rodd ZA, Bell RR, Ward RJ.** Is ethanol a pro-drug? Acetaldehyde contribution to brain ethanol effects. A*lcohol Clin Exp Res* 29: 1514–1521, 2005.
382. **Quintanilla ME, Israel Y, Sapag A, Tampier L.** The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. *Addict Biol* 11: 310–323, 2006.
383. **Rasmussen DD, Bryant CA, Boldt BM, Colasurdo EA, Levin N, Wilkinson CW.** Acute alcohol effects on opiomelanocortinergic regulation. *Alcohol Clin Exp Res* 22: 789–801, 1998.
384. **Rassnick S, D'Amico E, Riley E, Koob GF.** GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol Clin Exp Res* 17: 124–130, 1993.
385. **Rassnick S, Pulvirenti L, Koob GF.** Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens. *Psychopharmacology* 109: 92–98, 1992.
386. **Rassnick S, Stinus L, Koob GF.** The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. *Brain Res* 623: 16–24, 1993.
387. **Ray LA, Hutchison KE.** Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. *Arch Gen Psychiatry* 64: 1069–1077, 2007.
388. **Ray LA, Hutchison KE, MacKillop J, Miranda R Jr, Audette A, Swift R, Monti PM.** Effects of naltrexone during the descending limb of the blood alcohol curve. *Am J Addict* 17: 257–264, 2008.
389. **Ren H, Honse Y, Peoples RW.** A site of alcohol action in the fourth membrane-associated domain of the *N*-methyl-D-aspartate receptor. *J Biol Chem* 278: 48815–48820, 2003.
390. **Ren H, Salous AK, Paul JM, Lipsky RH, Peoples RW.** Mutations at F637 in the NMDA receptor NR2A subunit M3 domain influence agonist potency, ion channel gating and alcohol action. *Br J Pharmacol* 151: 749–757, 2007.
391. **Reppert SM, Weaver DR.** Coordination of circadian timing in mammals. *Nature* 418: 935–941, 2002.
392. **Rezvani AH, Grady DR, Peek AE, Pucilowski O.** Inhibition of nitric oxide synthesis attenuates alcohol consumption in two strains of alcohol-preferring rats. *Pharmacol Biochem Behav* 50: 265–270, 1995.
393. **Richter CP, Campbell KH.** Alcohol taste thresholds and concentrations of solution preferred by rats. *Science* 91: 507–508, 1940.
394. **Rimondini R, Arlinde C, Sommer W, Heilig M.** Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J* 16: 27–35, 2002.
395. **Rimondini R, Sommer WH, Dall'Olio R, Heilig M.** Long-lasting tolerance to alcohol following a history of dependence. *Addict Biol* 13: 26–30, 2008.

396. **Risinger FO, Freeman PA, Rubinstein M, Low MJ, Grandy DK.** Lack of operant ethanol self-administration in dopamine D2 receptor knockout mice. *Psychopharmacology* 152: 343–350, 2000.
397. **Risinger FO, Freeman PA, Greengard P, Fienberg AA.** Motivational effects of ethanol in DARPP-32 knockout mice. *J Neurosci* 21: 340–348, 2001.
398. **Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF.** Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacology* 22: 581–594, 2000.
399. **Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HW, Koob GF, Gold LH.** mu-Opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther* 293: 1002–1008, 2000.
400. **Robinson TE, Berridge KC.** The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev* 18: 247–291, 1993.
401. **Robledo P, Berrendero F, Ozaita A, Maldonado R.** Advances in the field of cannabinoid-opioid cross-talk. *Addict Biol* 13: 213–224, 2008.
402. **Rodd ZA, Melendez RI, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ.** Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for involvement of dopamine neurons. *J Neurosci* 24: 1050–1057, 2004.
403. **Rodd-Henricks ZA, McKinzie DL, Melendez RI, Berry N, Murphy JM, McBride WJ.** Effects of serotonin-3 receptor antagonists on the intracranial self-administration of ethanol within the ventral tegmental area of Wistar rats. *Psychopharmacology* 165: 252–259, 2003.
404. **Rodd ZA, Bertsch BA, Strother WN, Le-Niculescu H, Balaraman Y, Hayden E, Jerome RE, Lumeng L, Nurnberger JI Jr, Edenberg HJ, McBride WJ, Niculescu AB.** Candidate genes, pathways and mechanisms for alcoholism: an expanded convergent functional genomics approach. *Pharmacogenomics J* 6: 1–3, 2006.
405. **Rodd ZA, McKinzie DL, Bell RL, McQueen VK, Murphy JM, Schoepp DD, McBride WJ.** The metabotropic glutamate 2/3 receptor agonist LY404039 reduces alcohol-seeking but not alcohol self-administration in alcohol-preferring (P) rats. *Behav Brain Res* 171: 207–215, 2006.
406. **Roelofs SM.** Hyperventilation, anxiety, craving for alcohol: a subacute alcohol withdrawal syndrome. *Alcohol* 2: 501–505, 1985.
407. **Rollema H, Coe JW, Chambers LK, Hurst RS, Stahl SM, Williams KE.** Rationale, pharmacology and clinical efficacy of partial agonists of alpha4beta2 nACh receptors for smoking cessation. *Trends Pharmacol Sci* 28: 316–325, 2007.
408. **Ron D, Jurd R.** The "ups and downs" of signaling cascades in addiction. *Sci STKE* 309: re14, 2005.
409. **Ronald KM, Mirshahi T, Woodward JJ.** Ethanol inhibition of *N*-methyl-D-aspartate receptors is reduced by site-directed mutagenesis of a transmembrane domain phenylalanine residue. *J Biol Chem* 276: 44729–44735, 2001.
410. **Rosenbloom M, Sullivan EV, Pfefferbaum A.** Using magnetic resonance imaging and diffusion tensor imaging to assess brain damage in alcoholics. *Alcohol Res Health* 27: 146–152, 2003.
411. **Rossetti ZL, Hmaidan Y, Diana M, Gessa GL.** Lack of tolerance to ethanol-induced dopamine release in the rat ventral striatum. *Eur J Pharmacol* 231: 203–207, 1993.
412. **Russell RN, McBride WJ, Lumeng L, Li TK, Murphy JM.** Apomorphine and 7-OH DPAT reduce ethanol intake of P and HAD rats. *Alcohol* 13: 515–519, 1996.
413. **Ryabinin AE, Criado JR, Henriksen SJ, Bloom FE, Wilson MC.** Differential sensitivity of c-Fos expression in hippocampus and other brain regions to moderate and low doses of alcohol. *Mol Psychiatry* 2: 32–43, 1997.
414. **Saal D, Dong Y, Bonci A, Malenka RC.** Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 37: 577–582, 2003.
415. **Saba L, Bhave SV, Grahame N, Bice P, Lapadat R, Belknap J, Hoffman PL, Tabakoff B.** Candidate genes and their regulatory elements: alcohol preference and tolerance. *Mamm Genome* 17: 669–688, 2006.
416. **Saba L, Porcella A, Congeddu E, Colombo G, Peis M, Pistis M, Gessa GL, Pani L.** The R100Q mutation of the GABA(A) alpha(6) receptor subunit may contribute to voluntary aversion to ethanol in the sNP rat line. *Brain Res* 87: 263–270, 2001.
417. **Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M.** Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 573–585, 1998.
418. **Salamone JD.** Will the last person who uses the term "reward" please turn out the lights? Comments on processes related to reinforcement, learning, motivation and effort. *Addict Biol* 11: 43–44, 2006.
419. **Salimov RM, Salimova NB.** The alcohol deprivation effect in hybrid mice. *Drug Alcohol Depend* 32: 187–191, 1993.
420. **Salimov RM, Salimova NB, Shvets LN, Maisky AI.** Haloperidol administered subchronically reduces the alcohol-deprivation effect in mice. *Alcohol* 20: 61–68, 2000.
421. **Samochocki M, Zerlin M, Jostock R, Groot Kormelink PJ, Luyten WH, Albuquerque EX, Maelicke A.** Galantamine is an allosterically potentiating ligand of the human alpha4/beta2 nAChR. *Acta Neurol Scand Suppl* 176: 68–73, 2000.
422. **Samson HH, Tolliver GA, Haraguchi M, Hodge CW.** Alcohol self-administration: role of mesolimbic dopamine. *Ann NY Acad Sci* 654: 242–253, 1992.
423. **Sanchis-Segura C, Borchardt T, Vengeliene V, Zghoul T, Bachteler D, Gass P, Sprengel R, Spanagel R.** Involvement of the AMPA receptor GluR-C subunit in alcohol-seeking behavior and relapse. *J Neurosci* 26: 1231–1238, 2006.
424. **Sanchis-Segura C, Cline B, Jurd R, Rudolph U, Spanagel R.** Etomidate and propofol-hyposensitive $GABA_A$ receptor beta3(N265M) mice show little changes in acute alcohol sensitivity but enhanced tolerance and withdrawal. *Neurosci Lett* 416: 275–278, 2007.
425. **Sanchis-Segura C, Spanagel R.** Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. *Addict Biol* 11: 2–38, 2006.
426. **Saxonov S, Berg P, Brutlag DL.** A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci USA* 103: 1412–1417, 2006.
427. **Schmitt U, Waldhofer S, Weigelt T, Hiemke C.** Free-choice ethanol consumption under the influence of GABAergic drugs in rats. *Alcohol Clin Exp Res* 26: 457–462, 2002.
428. **Schneider ER, Rada P, Darby RD, Leibowitz SF, Hoebel BG.** Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. *Alcohol Clin Exp Res* 31: 1858–1865: 2007.
429. **Scholz H, Franz M, Heberlein U.** The hangover gene defines a stress pathway required for ethanol tolerance development. *Nature* 436: 845–847, 2005.
430. **Schubert F, Gallinat J, Seifert F, Rinneberg H.** Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. *Neuroimage* 21: 1762–1771, 2004.
431. **Schubert DS, Wolf AW, Patterson MB, Grande TP, Pendleton L.** A statistical evaluation of the literature regarding the associations among alcoholism, drug abuse, and antisocial personality disorder. *Int J Addict* 23: 797–808, 1988.
432. **Schuckit MA.** Genetics of the risk for alcoholism. *Am J Addict* 9: 103–112, 2000.
433. **Schultz W.** Multiple dopamine functions at different time courses. *Annu Rev Neurosci* 30: 259–288, 2007.
434. **Schumann G.** Okey Lecture 2006: identifying the neurobiological mechanisms of addictive behaviour. *Addiction* 102: 1689–1695, 2007.
435. **Schwarz AJ, Gozzi A, Reese T, Bifone A.** In vivo mapping of functional connectivity in neurotransmitter systems using pharmacological MRI. *Neuroimage* 34: 1627–1636, 2007.
436. **Schwarz AJ, Gozzi A, Reese T, Bifone A.** Functional connectivity in the pharmacologically activated brain: resolving networks of correlated responses to D-amphetamine. *Magn Reson Med* 57: 704–713, 2007.
437. **Schwarz AJ, Gozzi A, Reese T, Heidbreder CA, Bifone A.** Pharmacological modulation of functional connectivity: the corre-

lation structure underlying the phMRI response to D-amphetamine modified by selective dopamine D3 receptor antagonist SB277011A. *Magn Reson Imaging* 25: 811–820, 2007.
438. **Seitz D, Widmann U, Seeger U, Nagele T, Klose U, Mann K, Grodd W.** Localized proton magnetic resonance spectroscopy of the cerebellum in detoxifying alcoholics. *Alcohol Clin Exp Res* 23: 158–163, 1999.
439. **Selhub J.** Homocysteine metabolism. *Annu Rev Nutr* 19: 217–246, 1999.
440. **Sellers EM, Toneatto T, Romach MK, Somer GR, Sobell LC, Sobell MB.** Clinical efficacy of the 5-HT_3 antagonist ondansetron in alcohol abuse and dependence. *Alcohol Clin Exp Res* 18: 879–885, 1994.
441. **Shafer RA, Levant B.** The D_3 dopamine receptor in cellular and organismal function. *Psychopharmacology* 135: 1–16, 1998.
442. **Shaham Y, Shalev U, Lu L, De Wit H, Stewart J.** The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology* 168: 3–20, 2003.
443. **Shelton KL, Balster RL.** Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists. *Behav Pharmacol* 5: 441–450, 1994.
444. **Siegmund S, Vengeliene V, Singer MV, Spanagel R.** Influence of age at drinking onset on long-term ethanol self-administration with deprivation and stress phases. *Alcohol Clin Exp Res* 29: 1139–1145, 2005.
445. **Sillaber I, Rammes G, Zimmermann S, Mahal B, Zieglgänsberger W, Wurst W, Holsboer F, Spanagel R.** Enhanced and delayed stress-induced alcohol drinking in mice lacking functional CRH1 receptors. *Science* 296: 931–933, 2002.
446. **Siméon FG, Brown AK, Zoghbi SS, Patterson VM, Innis RB, Pike VW.** Synthesis and simple ^{18}F-labeling of 3-fluoro-5-(2-(2-(fluoromethyl)thiazol-4-yl)ethynyl) benzonitrile as a high affinity radioligand for imaging monkey brain metabotropic glutamate subtype-5 receptors with positron emission tomography. *J Med Chem* 50: 3256–3266, 2007.
447. **Simon EJ, Hiller JM, Edelmann I.** Stereospecific binding of the potent narcotic analgesic (^{3}H)-Etorphine to rat homogenate. *Proc Natl Acad Sci USA* 70: 1974–1949, 1973.
448. **Sinclair JD.** The alcohol-deprivation effect in monkeys. *Psychonom Sci* 25: 21–24, 1971.
449. **Slawecki CJ, Thorsell A, Ehlers CL.** Long-term neurobehavioral effects of alcohol or nicotine exposure in adolescent animal models. *Ann NY Acad Sci* 1021: 448–452, 2004.
450. **Smothers CT, Woodward JJ.** Effects of amino acid substitutions in transmembrane domains of the NR1 subunit on the ethanol inhibition of recombinant *N*-methyl-D-aspartate receptors. *Alcohol Clin Exp Res* 30: 523–530, 2006.
451. **Solomon RL, Corbit JD.** An opponent-process theory of motivation. I. Temporal dynamics of affect. *Psychol Rev* 81: 119–145, 1974.
452. **Sommer W, Arlinde C, Caberlotto L, Thorsell A, Hyytia P, Heilig M.** Differential expression of diacylglycerol kinase iota and L18A mRNAs in the brains of alcohol-preferring AA and alcohol-avoiding ANA rats. *Mol Psychiatry* 6: 103–108, 2001.
453. **Sommer W, Arlinde C, Heilig M.** The search for candidate genes of alcoholism: evidence from expression profiling studies. *Addict Biol* 10: 71–79, 2005.
454. **Sommer W, Hyytia P, Kiianmaa K.** The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking. *Addict Biol* 11: 289–309, 2006.
455. **Sommer W, Rimondini R, Marquitz M, Lidström J, Siems WE, Bader M, Heilig M.** Plasticity and impact of the central renin-angiotensin system during development of ethanol dependence. *J Mol Med* 85: 1089–1097, 2007.
456. **Sommer W, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig M.** Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala Crhr1 expression following a history of dependence. *Biol Psychiatry* 63: 139–145, 2008.
457. **Spanagel R, Heilig M.** Addiction and its brain science. *Addiction* 100: 1813–1822, 2005.
458. **Spanagel R, Herz A, Bals-Kubik R, Shippenberg TS.** Beta-endorphin-induced locomotor stimulation and reinforcement are associated with an increase in dopamine release in the nucleus accumbens. *Psychopharmacology* 104: 51–56, 1991.
459. **Spanagel R, Herz A, Shippenberg TS.** Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci USA* 89: 2046–2050, 1992.
460. **Spanagel R, Hölter S, Allingham K, Landgraf R, Zieglgänsberger W.** Acamprosate and alcohol. I. Effects on alcohol intake following alcohol deprivation in the rat. *Eur J Pharmacol* 305: 9–44, 1996.
461. **Spanagel R, Kiefer F.** Drugs for relapse prevention of alcoholism: ten years of progress. *Trends Pharmacol Sci* 29: 109–115, 2008.
462. **Spanagel R, Montkowski A, Allingham K, Stöhr T, Shoaib M, Holsboer F, Landgraf R.** Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. *Psychopharmacology* 122: 369–373, 1995.
463. **Spanagel R, Hölter SM.** Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? *Alcohol Alcohol* 34: 231–243, 1999.
464. **Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, Lascorz J, Depner M, Holzberg D, Soyka M, Schreiber S, Matsuda F, Lathrop M, Schumann G, Albrecht U.** The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. *Nat Med* 11: 35–42, 2005.
465. **Spanagel R, Rosenwasser AM, Schumann G, Sarkar DK.** Alcohol consumption and the body's biological clock. *Alcohol Clin Exp Res* 29: 1550–1557, 2005.
466. **Spanagel R, Sigmund S, Cowen M, Schroff KC, Schumann G, Fiserova M, Sillaber I, Wellek S, Singer MV, Putzke J.** The neuronal nitric oxide synthase (nNOS) gene is critically involved in neurobehavioral effects of alcohol. *J Neurosci* 22: 8676–8683, 2002.
467. **Spanagel R, Weiss F.** The dopamine hypothesis of reward: past and current status. *Trends Neurosci* 22: 521–527, 1999.
468. **Spanagel R, Zieglgänsberger W.** Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. *Trends Pharmacol Sci* 18: 54–59, 1997.
469. **Spear LP, Varlinskaya EI.** Adolescence. Alcohol sensitivity, tolerance and intake. *Recent Dev Alcohol* 17: 143–159, 2005.
470. **Srisurapanont M, Jarusuraisin N.** Opioid antagonists for alcohol dependence. *Cochrane Database of Systematic Reviews*, Issue 3. Art. No.: CD001867. DOI: 10.1002/14651858.CD001867.pub2, 2000.
471. **Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE.** Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci USA* 104: 12518–12523, 2007.
472. **Stephens DN, Brown G.** Disruption of operant oral self-administration of ethanol, sucrose, and saccharin by the AMPA/kainate antagonist, NBQX, but not the AMPA antagonist, GYKI 52466. *Alcohol Clin Exp Res* 23: 1914–1920, 1999.
473. **Stewart RB, Gatto GJ, Lumeng L, Li TK, Murphy JM.** Comparison of alcohol-preferring (P) and nonpreferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. *Alcohol* 10: 1–10, 1993.
474. **Steward CA, Marsden CA, Prior MJ, Morris PG, Shah YB.** Methodological considerations in rat brain BOLD contrast pharmacological MRI. *Psychopharmacology* 180: 687–704, 2005.
475. **St. Laurent G III, Wahlestedt C.** Noncoding RNAs: couplers of analog and digital information in nervous system function? *Trends Neurosci* 30: 612–621, 2007.
476. **Stolerman IP.** Drugs of abuse: behavioral principles, methods and terms. *Trends Pharmacol Sci* 13: 170–175, 1991.
477. **Stuber GD, Hopf FW, Hahn J, Cho SL, Guillory A, Bonci A.** Voluntary ethanol intake enhances excitatory synaptic strength in the ventral tegmental area. *Alcohol Clin Exp Res* 2008.
478. **Sullivan PF, Fan C, Perou CM.** Evaluating the comparability of gene expression in blood and brain. *Am J Genet B Neuropsychiatr Genet* 141: 261–268, 2006.
479. **Sullivan RJ, Hagen EH.** Psychotropic substance-seeking: evolutionary pathology or adaptation? *Addiction* 97: 389–400, 2002.
480. **Sumner BE, Cruise LA, Slattery DA, Hill DR, Shahid M, Henry B.** Testing the validity of c-*fos* expression profiling to aid the therapeutic classification of psychoactive drugs. *Psychopharmacology* 171: 306–321, 2004.

481. **Swendsen JD, Merikangas KR, Canino GJ, Kessler RC, Rubio-Stipec M, Angst J.** The comorbidity of alcoholism with anxiety and depressive disorders in four geographic communities. *Compr Psychiatry* 39: 176–184, 1998.
482. **Szumlinski KK, Ary AW, Lominac KD.** Homers regulate drug-induced neuroplasticity: implications for addiction. *Biochem Pharmacol* 75: 112–133, 2008.
483. **Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, Meloy MJ, Brown SA.** Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Arch Gen Psychiatry* 60: 727–735, 2003.
484. **Tarantino LM, McClearn GE, Rodrigues LA, Plomin R.** Confirmation of quantitative trait loci for alcohol preference in mice. *Alcohol Clin Exp Res* 22: 1099–1105, 1998.
485. **Templin MF, Stoll D, Schwenk JM, Potz O, Kramer S, Joos TO.** Protein microarrays: promising tools for proteomic research. *Proteomics* 3: 2155–2166, 2003.
486. **Terenius L.** Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol Toxicol* 32: 317–320, 1973.
487. **Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA, Heidbreder CA, Melotto S.** Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. *Addict Biol* 12: 22–29, 2007.
488. **Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND.** Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behav Brain Res* 164: 206–213, 2005.
489. **Thanos PK, Katana JM, Ashby CR Jr, Michaelides M, Gardner EL, Heidbreder CA, Volkow ND.** The selective dopamine D3 receptor antagonist SB-277011-A attenuates ethanol consumption in ethanol preferring (P) and non-preferring (NP) rats. *Pharmacol Biochem Behav* 81: 190–197, 2005.
490. **Thiele TE, Marsh DJ, Ste Marie L, Bernstein IL, Palmiter RD.** Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature* 396: 366–369, 1998.
491. **Thiele TE, Willis B, Stadler J, Reynolds JG, Bernstein IL, McKnight GS.** High ethanol consumption and low sensitivity to ethanol-induced sedation in protein kinase A-mutant mice. *J Neurosci* 20: RC75–78, 2000.
492. **Thorberg FA, Lyvers M.** Negative mood regulation (NMR) expectancies, mood, and affect intensity among clients in substance disorder treatment facilities. *Addict Behav* 31: 811–820, 2006.
493. **Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W.** Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nat Genet* 19: 162–166, 1998.
494. **Tonstad S, Tønnesen P, Hajek P, Williams KE, Billing CB, Reeves KR; Varenicline Phase 3 Study Group.** Effect of maintenance therapy with varenicline on smoking cessation: a randomized controlled trial. *JAMA* 296: 64–71, 2006.
495. **Treutlein J, Kissling C, Frank J, Wiemann S, Dong L, Depner M, Saam C, Lascorz J, Soyka M, Preuss UW, Rujescu D, Skowronek MH, Rietschel M, Spanagel R, Heinz A, Laucht M, Mann K, Schumann G.** Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. *Mol Psychiatry* 11: 594–602, 2006.
496. **Tsai G, Coyle JT.** The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. *Annu Rev Med* 49: 173–184, 1998.
497. **Tsankova N, Renthal W, Kumar A, Nestler EJ.** Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 8: 355–367, 2007.
498. **Tu Y, Kroener S, Abernathy K, Lapish C, Seamans J, Chandler LJ, Woodward JJ.** Ethanol inhibits persistent activity in prefrontal cortical neurons. *J Neurosci* 27: 4765–4775, 2007.
499. **Tzschentke TM.** Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol* 12: 227–462, 2007.
500. **Uddin RK, Singh SM.** Ethanol-responsive genes: identification of transcription factors and their role in metabolomics. *Pharmacogenomics J* 7: 38–47, 2007.
501. **Ungless MA, Whistler JL, Malenka RC, Bonci A.** Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 411: 583–587, 2001.
502. **Vagts AJ, He DY, Yaka R, Ron D.** Cellular adaptation to chronic ethanol results in altered compartmentalization and function of the scaffolding protein RACK1. *Alcohol Clin Exp Res* 27: 1599–1605, 2003.
503. **Valverde O, Mantamadiotis T, Torrecilla M, Ugedo L, Pineda J, Bleckmann S, Gass P, Kretz O, Mitchell JM, Schutz G, Maldonado R.** Modulation of anxiety-like behavior and morphine dependence in CREB-deficient mice. *Neuropsychopharmacology* 29: 1122–1133, 2004.
504. **Van der Kooy D, Mucha RF, O'Shaughnessy M, Bucenieks P.** Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. *Brain Res* 243: 107–117, 1982.
505. **Van Ree JM, Smyth DG, Colpaert FC.** Dependence creating properties of lipotropin C-fragment (β-endorphin): evidence for its internal control of behaviour. *Life Sci* 24: 495–502, 1979.
506. **Vengeliene V, Bachteler D, Danysz W, Spanagel R.** The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. *Neuropharmacology* 48: 822–829, 2005.
507. **Vengeliene V, Bilbao A, Molander A, Spanagel R.** Neuropharmacology of alcohol addiction. *Br J Pharmacol* 154: 299–315, 2008.
508. **Vengeliene V, Heidbreder CA, Spanagel R.** The effects of lamotrigine on alcohol seeking and relapse. *Neuropharmacology* 53: 951–957, 2007.
509. **Vengeliene V, Leonardi-Essmann F, Perreau-Lenz S, Gebicke-Haerter P, Drescher K, Gross G, Spanagel R.** The dopamine D3 receptor plays an essential role in alcohol-seeking and relapse. *FASEB J* 20: 2223–2233, 2006.
510. **Vengeliene V, Vollmayer B, Henn FA, Spanagel R.** Alcohol self-administration in two rat lines selectively bred for helplessness and non-helplessness behavior. *Psychopharmacology* 178: 125–132, 2005.
511. **Viglinskaya IV, Overstreet DH, Kashevskaya OP, Badishtov BA, Kampov-Polevoy AB, Seredenin SB, Halikas JA.** To drink or not to drink: tests of anxiety and immobility in alcohol-preferring and -nonpreferring rat strains. *Physiol Behav* 57: 937–941, 1995.
512. **Villarroya M, García AG, Marco-Contelles J, López MG.** An update on the pharmacology of galantamine. *Expert Opin Invest Drugs* 16: 1987–1998, 2007.
513. **Volkow ND, Li TK.** Drug addiction: the neurobiology of behaviour gone awry. *Nat Rev Neurosci* 5: 963–970, 2004.
514. **Volkow ND, Wang GJ, Begleiter H, Porjesz B, Fowler JS, Telang F, Wong C, Ma Y, Logan J, Goldstein R, Alexoff D, Thanos PK.** High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. *Arch Gen Psychiatry* 63: 999–1008, 2006.
515. **Volkow ND, Wang GJ, Maynard L, Fowler JS, Jayne B, Telang F, Logan J, Ding YS, Gatley SJ, Hitzemann R, Wong C, Pappas N.** Effects of alcohol detoxification on dopamine D2 receptors in alcoholics: a preliminary study. *Psychiatry Res* 116: 163–172, 2002.
516. **Vollmayr B, Bachteler D, Vengeliene V, Gass P, Spanagel R, Henn FA.** Rats with congential learned helplessness respond less to sucrose but show no deficits in activity or learning. *Behav Brain Res* 150: 217–221, 2004.
517. **Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP.** Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry* 49: 876–880, 1992.
518. **Walker BM, Koob GF.** Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence. *Neuropsychopharmacology* 33: 643–652, 2008.
519. **Wallner M, Hanchar HJ, Olsen RW.** Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc Natl Acad Sci USA* 100: 15218–15223, 2003.
520. **Wand G, Levine M, Zweifel L, Schwindinger W, Abel T.** The cAMP-protein kinase A signal transduction pathway modulates

ethanol consumption and sedative effects of ethanol. *J Neurosci* 21: 5297–5303, 2001.
521. **Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G.** Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. *Proc Natl Acad Sci USA* 100: 1393–1398, 2003.
522. **Wang X, Wang G, Lemos JR, Treistman SN.** Ethanol directly modulates gating of a dihydropyridine-sensitive Ca^{2+} channel in neurohypophysial terminals. *J Neurosci* 14: 5453–5460, 1994.
523. **Wegelius K, Honkanen A, Korpi ER.** Benzodiazepine receptor ligands modulate ethanol drinking in alcohol-preferring rats. *Eur J Pharmacol* 263: 141–147, 1994.
524. **Weiss F, Lorang MT, Bloom FE, Koob GF.** Ethanol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *J Pharmacol Exp Ther* 267: 250–258, 1993.
525. **Weissman MM, Olfson M.** Depression in women: implications for health care research. *Science* 269: 799–801, 1995.
526. **Weitlauf C, Egli RE, Grueter BA, Winder DG.** High-frequency stimulation induces ethanol-sensitive long-term potentiation at glutamatergic synapses in the dorsolateral bed nucleus of the stria terminalis. *J Neurosci* 24: 5741–5747, 2004.
527. **Werner C, Raivich G, Cowen M, Strekalova T, Sillaber I, Spanagel R, Hofmann F.** Importance of NO/cGMP signalling via cGMP-dependent protein kinase II for mediating emotionality. *Eur J Neurosci* 20: 3498–3506, 2004.
528. **Weston AD, Hood L.** Systems biology, proteomics, and the future of health care: toward predictive, preventative, and personalized medicine. *J Proteome Res* 3: 179–196, 2004.
529. **White AM, Swartzwelder HS.** Hippocampal function during adolescence: a unique target of ethanol. *Ann NY Acad Sci* 1021: 206–220, 2004.
530. **WHO.** *Global Status Report on Alcohol and Drugs of Abuse*, 2004.
531. **Wiens F, Zitzmann A, Lachance MA, Yegles M, Pragst F, Wurst FM, von Holst D, Guan SL, Spanagel R.** Chronic intake of fermented floral nectar by wild treeshrews. *Proc Natl Acad Sci USA* 105: 10426–10431, 2008.
532. **Winstanley CA.** The orbitofrontal cortex, impulsivity, and addiction: probing orbitofrontal dysfunction at the neural, neurochemical, and molecular level. *Ann NY Acad Sci* 1121: 639–655, 2007.
533. **Wise RA.** Dopamine, learning and motivation. *Nat Rev Neurosci* 5: 483–494, 2004.
534. **Wise RA, Rompre PP.** Brain dopamine and reward. *Annu Rev Psychol* 40: 191–225, 1989.
535. **Wolffgramm J, Heyne A.** From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. *Behav Brain Res* 70: 77–94, 1995.
536. **Wright JM, Peoples RW, Weight FF.** Single-channel and whole-cell analysis of ethanol inhibition of NMDA-activated currents in cultured mouse cortical and hippocampal neurons. *Brain Res* 738: 249–256, 1996.
537. **Yamaguchi T, Sheen W, Morales M.** Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci* 25: 106–118, 2007.
538. **Yamamoto T, Nakahata Y, Tanaka M, Yoshida M, Soma H, Shinohara K, Yasuda A, Mamine T, Takumi T.** Acute physical stress elevates mPeriod1 mRNA expression in mouse peripheral tissues via a glucocorticoid responsive element. *J Biol Chem* 280: 42036–42043, 2005.
539. **Yao L, Arolfo MP, Dohrman DP, Jiang Z, Fan P, Fuchs S, Janak PH, Gordon AS, Diamond I.** $\beta\gamma$ Dimers mediate synergy of dopamine D2 and adenosine A2 receptor-stimulated PKA signaling and regulate ethanol consumption. *Cell* 109: 733–743, 2002.
540. **Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ.** Increase in plasma homocysteine associated with parallel increases in plasma *S*-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J Biol Chem* 275: 29318–29323, 2000.
541. **Yin HH, Park BS, Adermark L, Lovinger DM.** Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum. *Eur J Neurosci* 25: 3226–3232, 2007.
542. **Yoder JA, Yen RW, Vertino PM, Bestor TH, Baylin SB.** New 5' regions of the murine and human genes for DNA (cytosine-5)-methyltransferase. *J Biol Chem* 271: 31092–31097, 1996.
543. **Yoshimoto K, McBride WJ, Lumeng L, Li TK.** Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. *Alcohol Clin Exp Res* 16: 781–785, 1992.
544. **Yücel M, Lubman DI, Harrison BJ, Fornito A, Allen NB, Wellard RM, Roffel K, Clarke K, Wood SJ, Forman SD, Pantelis C.** A combined spectroscopic and functional MRI investigation of the dorsal anterior cingulate region in opiate addiction. *Mol Psychiatry* 12: 691–702, 2007.
545. **Zhou FC, McKinzie DL, Patel TD, Lumeng L, Li TK.** Additive reduction of alcohol drinking by 5-HT_{1A} antagonist WAY 100635 and serotonin uptake blocker fluoxetine in alcohol-preferring P rats. *Alcohol Clin Exp Res* 22: 266–269, 1998.
546. **Zhou Z, Zhu G, Hariri AR, Enoch MA, Scott D, Sinha R, Virkkunen M, Mash DC, Lipsky RH, Hu XZ, Hodgkinson CA, Xu K, Buzas B, Yuan Q, Shen PH, Ferrell RE, Manuck SB, Brown SM, Hauger RL, Stohler CS, Zubieta JK, Goldman D.** Genetic variation in human NPY expression affects stress response and emotion. *Nature* 452: 997–1001, 2008.
547. **Zimmermann P, Wittchen HU, Höfler M, Pfister H, Kessler RC, Lieb R.** Primary anxiety disorders and the development of subsequent alcohol use disorders: a 4-year community study of adolescents and young adults. *Psychol Med* 33: 1211–1222, 2003.
548. **Zimmermann US, Blomeyer D, Laucht M, Mann KF.** How gene-stress-behavior interactions can promote adolescent alcohol use: the roles of predrinking allostatic load and childhood behavior disorders. *Pharmacol Biochem Behav* 86: 246–262, 2007.
549. **Zimmermann US, Mick I, Vitvitskyi V, Plawecki MH, Mann KF, O'Connor S.** Development and pilot validation of Computer-Assisted Self-infusion of Ethanol (CASE): a new method to study alcohol self-administration in humans. *Alcohol Clin Exp Res* 32: 1321–1328, 2008.

Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior

Rainer Spanagel

Physiol Rev 89:649-705, 2009. doi:10.1152/physrev.00013.2008

Physiological Reviews provides state of the art coverage of timely issues in the physiological and biomedical sciences. It is published quarterly in January, April, July, and October by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2009 by the American Physiological Society. ISSN: 0031-9333, ESSN: 1522-1210. Visit our website at http://www.the-aps.org/.