# Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior

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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). 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*) thrive in this million-year-old ecosystem, the pentailed tree shrew has adapted to a daily intake of intoxicating amounts of alcohol on a daily basis (531). Pentaileld 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 a 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
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. 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

\[\text{FIG. 1. Alcohol consumption per capita in liters of pure ethanol.}\]
inwardly rectifying K
hit of alcohol on specific targets in the brain, a second
Well as L-type Ca$^{2+}$

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-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 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

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

<table>
<thead>
<tr>
<th>Criteria for Alcohol Dependence</th>
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<tr>
<td>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:</td>
</tr>
<tr>
<td>1. Tolerance</td>
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<tr>
<td>2. Withdrawal</td>
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<tr>
<td>3. Alcohol is often taken in larger amounts or over a longer period than was intended</td>
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<td>4. There is a persistent desire or there are unsuccessful efforts to cut down or control alcohol use</td>
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<td>5. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects</td>
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<tr>
<td>6. Important social, occupational, or recreational activities are given up or reduced because of alcohol use</td>
</tr>
<tr>
<td>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)</td>
</tr>
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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).
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). 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. In a key publication, David Lovinger et al. (283) demonstrated that

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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

![NMDA Receptor Subunit Topology Diagram](image)

**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_\text{A} receptors is enhanced by ethanol. The GABA_\text{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_\text{A} receptors being composed of \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\)-subunits (23). With the use of different receptor constructs, a region in the TM domains of the \(\alpha/\beta\) subunits of the GABA_\text{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_\text{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-HT_3 receptor function (282, 289). The 5-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_\text{A} receptors strongly depends on the subunit composition. While most subunit compositions of GABA_\text{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_\text{A} receptors containing \(\delta\) 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 \(\beta\) subunit do not differ in their acute response to ethanol when compared with wild-type animals (424). These findings suggest that “extrasynaptic” \(\delta\) subunit-containing GABA_\text{A} receptors (without a prominent role of the associated \(\beta3\) subunit), but not their “synaptic” \(\gamma\) 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-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-

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\(^5\)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-HT_2A_ 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 experimenter 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-
onists and certain GABA-mimetic drugs acting through different sites within the GABA<sub>A</sub> 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-HT<sub>3</sub> 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<sub>A</sub> 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), 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...
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 somatodendritic 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 secretagoge 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 4C). 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 chromosome 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).5

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 Finnish alcohol-prefering AA rats and corresponding alcohol-avoiding ANA rats (454) has been reported by Kianmäa 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 experimenters-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-

5 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·kg−1·day−1 ethanol, whereas the nonpreferring lines consume less than 1 g·kg−1·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 [11C]raclopride binding in the NAC in healthy volunteers after alcohol ingestion. In this study the magnitude of the change in [11C]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 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 non-preferring 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 somatodendritic 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 serotoninergic 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 glycinerergic 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 glycinergetic 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-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 its 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. 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 acquisition 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 (Gαs). Alternatively, D2-like receptors (D2-D4) inhibit AC through inhibitory Gαi. 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 Ca2+/calmodulin-dependent protein kinase IV (CaMKIV) and CREB phosphorylation, specifically in the shell of NAC (322), suggesting that decreased CaMKIV-dependent CREB phosphorylation in
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).

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 G\(_{\beta\gamma}\) 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 \(\beta\gamma\)-catalytic subunit of PKA (PKA-C\(_{\beta\gamma}\)) 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 dopaminergic neurons that contain D1 receptors along with the DARPP-32/PP1 cascade. This cascade 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 \(\beta\)-arrestin 2 (43). The two isoforms PKC-\(\epsilon\) and PKC-\(\gamma\) interact with these scaffolding proteins, and they seem to be of particular importance in mediating alcohol-induced behavioral responses. PKC-\(\gamma\) knockout mice show enhanced alcohol preference (62) compared with wild-type mice, whereas PKC-\(\epsilon\) knockouts exhibit a markedly reduced preference for alcohol (192). The latter phenotype could be rescued by means of inducible expression of PKC-\(\epsilon\) in the NAC, and other forebrain areas restored alcohol preference in adult PKC-\(\epsilon\) knockout mice to the level seen in wild-type mice (81). These findings indicate that PKC-\(\epsilon\) 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-\(\gamma\) knockout mice (177) or augmented function in PKC-\(\epsilon\) 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-prefering 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

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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<sub>α</sub> receptor function. Hence, alcohol affects the functioning of receptors (NMDA and GABA<sub>α</sub>) 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, 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 non-coding 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

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.
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 lacZ under the control of CRE. With the use of histochemistry to map CRE-mediated gene transcription in the brain of CRE-lacZ 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 angiogenesis seen in the prefrontal cortex of chronically ethanol-exposed rats as well as of alcohol-prefering 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, 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 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 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...
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 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 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 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.

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. 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-prefering msP rats before and after ethanol access compared with naïve, 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 naïve 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 naïve Wistar, respectively. Open circle, no visible difference; red circle, for comparison, difference in Chr1 expression between naïve and ethanol-drinking msP is $P < 0.01$ (171). Genes: Crh, corticosterone-releasing hormone; Chr1, corticosterone-releasing hormone receptor type 1; Pdn, prodynorphin; Opr1, 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.)
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, Ca2+-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 NMDA-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 only has 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. VII). 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 relationships in the etiology of alcoholism. IMAGEN is the first study of amygdala activation in subjects with various genetic variants 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 anticonvulsants (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 [11C]raclopride. They found a significant reduction in [11C]raclopride binding potential in the NAC, indicative of increased extracellular DA. The magnitude of the change in [11C]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-
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). 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 [11C]raclopride PET to assess DA D2 receptor and [18F]fluodeoxyglucose 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).

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 11C-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 identifying circuits involved in the inhibition of behavioral response and the control of emotion (514).

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).

FIG. 10. Brain imaging of central μ-opioid receptor availability with a [11C]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-opioid receptor availability and severity of alcohol craving [Obsessive Compulsive Drinking Scale (OCDS) score] in alcohol-dependent patients. (Figure kindly provided by K. Mann.)
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 [1H]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 neuro-metabolic 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-
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). 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

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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 relapse-like 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). 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, the ADE in rats was induced by addition of 5% 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. 8.3.6).

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. 10).

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-
tion, i.e., the animal is tested under conditions of non-reinforcement 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 individual’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-prefering rat lines would, therefore, be predicted to be more anxious than their nonpreferring counterparts. Although this holds true for Sardinian alcohol-prefering (93) and Marchigian Sardinian alcohol-prefering rats (83), Indianapolis P-rats are less anxious than their nonpreferring counterparts (473). When all the comparative studies between multiple alcohol-prefering and nonpreferring lines are taken into consideration, the hypothesis that alcohol-prefering 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 different 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-prefer-
ing 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-prefering 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 increased 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-prefering 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 (hCRH1) 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 hCRH1 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 hCRH1 and an association of these genetic variations of the hCRH1 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 hCRH1 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 hCRH1 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 Per1 gene 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
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, 105, 405, 423, 464, 506; agents acting on GABA receptors, Refs. 70, 95, 427. (Figure produced by Valentina Vengeliene.)

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Fig. 12.—Continued
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
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-

![Agent acting on dopamine receptors (details are as in Fig. 12); see Refs. 277, 420, 509. (Figure produced by Valentina Vengeliene.]

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

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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

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<td>Nociceptin (orphanin FQ), opioid-like nociceptin receptor endogenous ligand</td>
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**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.)
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-HT₃ antagonist ondansetron is another promising medication for the treatment of alcohol addiction. As outlined in section II, the 5-HT₃ 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-
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 α4β2 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 α4β2 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. Galantamine 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 galantamine 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 α4β2 plays a pivotal role. Partial agonists, which act on α4β2 containing nACh receptors, have been designed as novel treatments for tobacco addiction. Such agents are thought to exert a dual effect by stimulating α4β2-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 α4β2-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
C. Individualized Pharmacotherapy for Alcoholism

Despite the efficacy of combined behavioral interventions and novel pharmacotherapies, 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 (20). 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, 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-prefering 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...
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<sub>A</sub>, glycine, 5-HT<sub>3</sub>, and nACh receptors, as well as L-type Ca<sup>2+</sup> channels and G protein-activated inwardly rectifying K<sup>+</sup> 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<sub>A</sub> 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 multi-electrode 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.

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