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REVIEW

Neuropharmacology of alcohol addiction

V Vengeliene, A Bilbao, A Molander and R Spanagel

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

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 NMDA, $GABA_A$, glycine, 5-hydroxytryptamine 3 (serotonin) and nicotinic ACh receptors as well as L-type Ca^{2+} channels and G-protein-activated inwardly rectifying K^+ channels. Following this 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 that leads subsequently to the typical acute behavioural effects of alcohol, ranging from disinhibition to sedation and even hypnosis, with increasing concentrations of alcohol. Besides these acute pharmacodynamic aspects of alcohol, we discuss the neurochemical substrates that are involved in the initiation and maintenance phase of an alcohol drinking behaviour. Finally, addictive behaviour towards alcohol as measured by alcohol-seeking and relapse behaviour is reviewed in the context of specific neurotransmitter/neuropeptide systems and their signalling pathways. The activity of the mesolimbic dopaminergic system plays a crucial role during the initiation phase of alcohol consumption. Following long-term, chronic alcohol consumption virtually all brain neurotransmission seems to be affected, making it difficult to define which of the systems contributes the most to the transition from controlled to compulsive alcohol use. However, compulsive alcohol drinking is characterized by a decrease in the function of the reward neurocircuitry and a recruitment of antireward/stress mechanisms comes into place, with a hypertrophic corticotropin-releasing factor system and a hyperfunctional glutamatergic system being the most important ones.
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Abbreviations: AA, Alko alcohol rat line; ADE, alcohol deprivation effect; AMPA, DL-α-amino-3-hydroxy-5-methylsoxazole-4-propionate; ANA, Alko non-alcohol rat line; CB, cannabinoid; CRF, corticotropin-releasing factor/hormone; DA, 3,4-dihydroxyphenylethylamine (dopamine); GIRKs, G-protein-activated inwardly rectifying K^+ channels; GluR1–4 (GluRA–D), AMPA receptor subunits 1, 2, 3, 4 (A, B, C, D); HAD, high-alcohol-drinking rat line; 5-HT, 5-hydroxytryptamine (serotonin); LORR, loss of righting reflex; mGluR, metabotropic glutamate receptor; NAC, nucleus accumbens; nAChR, nicotinic ACh receptor; NMDAR, NMDA receptor; NO, nitric oxide; NPY, neuropeptide Y; NR1, NMDA receptor subunit 1; NR2A–D, NMDA receptor subunits 2A, 2B, 2C and 2D; P, iP, alcohol-preferring rat lines; SNP, Sardinian alcohol non-preferring; SP, Sardinian alcohol-preferring rat line; VTA, ventral tegmental area

Introduction

Depending on various modulating factors, such as genetic predisposition, provocative environmental experiences, social context, pharmacological history and others, alcohol consumption can become compulsive, and finally an addictive behaviour might evolve. However, despite remarkable progress in the elucidation of susceptibility factors contributing to the development of alcohol addiction, the exact mechanism(s) of this phenomenon remain(s) to be elucidated. In particular, there are two main questions that need to be answered: (i) what are the factors (genetic or environmental) that are involved in the initiation and maintenance of alcohol consumption? and (ii) what are the changes in the brain that underlie the transition from controlled to compulsive alcohol use?

Genetically determined nonspecific susceptibility factors that substantially increase the risk to develop alcohol addiction include concomitant psychiatric disorders, such as anxiety and major depressive disorders. There are also known personality traits, such as passive dependent, impulsive or antisocial traits that lead to a person's differential response to novelty, punishment and reward, and to adaptive responses to environmental challenges in general (Cloninger, 1987). These psychiatric disorders and

Correspondence: Dr R Spanagel, Department of Psychopharmacology, Zentralinstitut für Seelische Gesundheit, University of Heidelberg, J5, Mannheim 68159, Germany.
E-mail: rainer.spanagel@zi-mannheim.de
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personality traits are supposed to reflect differences in brain neurotransmitter systems that in turn influence the pharmacodynamics of alcohol and determine, at least in part, an individual's liability in seeking alcohol reward and to become dependent on it after long-term and excessive exposure.

In addition, there are a number of mechanisms that determine the pharmacokinetics of alcohol. It is well known that genes that have a major effect on alcohol metabolism influence the reaction to alcohol and the risk to develop alcohol addiction. Thus, alcohol dehydrogenase and aldehyde dehydrogenase are two variants that can cause the aversive flushing reaction following alcohol intake and can thereby protect the individual from further alcohol consumption. Acetaldehyde, which is the first product generated during alcohol metabolism, is not only involved in mediating the flushing reaction but might also induce some effects that mimic those of alcohol. Acetaldehyde can be produced by the enzyme catalase within the brain after alcohol administration (Aragon *et al*., 1991), and studies in which the activity of catalase was manipulated suggest that acetaldehyde, especially its stimulant properties, contributes to many behavioural effects of alcohol (Sanchis-Segura *et al*., 2005). Acetaldehyde affects the activity of different neurotransmitter systems unselectively, thus its mechanism of action is not clear. Current evidence suggests that the contribution of acetaldehyde to alcohol's effects is best explained by a process in which acetaldehyde modulates, rather than mediates, some of alcohol's effects (Quertemont *et al*., 2005).

Alcohol addiction is a complex disorder and cannot be modelled in animals as a whole. However, the initiation and maintenance of alcohol consumption, and alcohol seeking during abstinence and relapse-like drinking can be successfully mimicked or modelled in laboratory animals (Sanchis-Segura and Spanagel, 2006). Thus, in the following sections we will review each phase of the alcohol addiction cycle separately and will discuss the neurotransmitter and neuropeptide systems involved in the initiation, maintenance and in the compulsive phase (consisting of alcohol-seeking and relapse behaviour). However, in the first section we will describe the first hit of alcohol on specific targets within the CNS.

Pharmacodynamic effects of alcohol

Alcohol has a complex pharmacology and acts by disrupting distinct receptor or effector proteins via direct or indirect interactions, whereas at very high concentrations it might even change the composition of lipids in the surrounding membrane. At concentrations in the 5–20 mM range—which constitutes the legal intoxication range for driving in many countries—alcohol directly interferes with the function of several ion channels and receptors.

In a key publication, Lovinger *et al*. (1989) showed that NMDA function was inhibited by alcohol in a concentration-dependent manner over the range from 5 to 50 mM, a range that also produces intoxication. The amplitude of the NMDA-activated current was reduced 61% by 50 mM alcohol. What is more, the potency of several alcohols to inhibit the NMDA-activated current is linearly related to their intoxicating potency, suggesting that alcohol-induced inhibition of responses to NMDA receptor activation may contribute to the neural and cognitive impairments associated with intoxication. In subsequent years, several other ionotropic receptors were characterized as primary targets of the action of alcohol. Thus, the function of $GABA_A$ and glycine receptors is enhanced by alcohol. By using chimaeric receptor constructs, a region of 45 amino-acid residues that is both necessary and sufficient for the enhancement of $GABA_A$ receptor function by alcohol has been identified (Mihic *et al*., 1997). In addition to its effects on $GABA_A$ and glycine receptors, alcohol potentiates 5-hydroxytryptamine 3 (serotonin) ($5\text{-}HT_3$) (Lovinger, 1999) and neuronal nicotinic ACh receptor (nAChR) function (Narahashi *et al*., 1999).

Ion channels also constitute a primary target of alcohol. Thus, alcohol inhibits dihydropyridine-sensitive L-type Ca^{2+} channels, and single-channel recordings suggest that alcohol's effects on gating are consistent with the interaction of a single drug molecule with a single target site, possibly the L-channel itself (Wang *et al*., 1994). In addition, alcohol opens G-protein-activated inwardly rectifying K^+ channels (GIRKs) (Kobayashi *et al*., 1999; Lewohl *et al*., 1999). Selective enhancement of GIRK2 function by intoxicating concentrations of alcohol was demonstrated for homomeric and heteromeric channels, and a region of 43 amino acids in the carboxyl terminus has been identified that is critical for the action of alcohol on these channels (Kobayashi *et al*., 1999; Lewohl *et al*., 1999).

These primary inhibitory and facilitatory actions of alcohol on ion channels and receptors depend on a number of variables, especially the concentration of alcohol and the subunit composition of a particular channel or receptor. For example, alcohol's action on $GABA_A$ receptors strongly depends on the subunit composition. $GABA_A$ receptors are composed of α, β, γ and δ subunits forming a pentameric ligand-gated ion channel receptor. Whereas most subunit compositions of $GABA_A$ receptors display responses to alcohol only at high concentrations (>60 mM), it has been found that very low concentrations (1–3 mM) of alcohol do alter the activity of $GABA_A$ receptors containing δ subunits. These extrasynaptic GABA receptors are exclusively associated *in vivo* with α4/α6 subunits and the β3 subunit. Moreover, in α4βδ subunit combinations, receptors containing the β3 subunit have been found to be almost 10 times more sensitive than receptors containing the β2 subunit, suggesting that the β3 subunit also constitutes an alcohol-sensitive site (Wallner *et al*., 2003). However, mouse models in which either the β3 subunit was genetically deleted or knock-in mice that carry a single-point mutation (*N265M*) in the β3 subunit do not differ in the acute response to alcohol when compared to wild-type animals (Sanchis-Segura *et al*., 2007). These combined findings suggest that 'extrasynaptic' $GABA_A$ receptors that contain δ subunits (without a prominent role of the associated β3 subunit) are primary targets for alcohol, as opposed to their 'synaptic' counterparts that contain γ subunits.

Also critical in the response to alcohol is the subunit composition of NMDA and other receptors. Thus, NMDA

receptors composed of either NMDA receptor NR1/NR2A or NR1/NR2B subunits are more sensitive to alcohol's inhibitory effects than those composed of NR1/NR2C or NR1/NR2D subunits (Allgaier, 2002). Similarly, glycine receptors containing α1 seem to be more sensitive to low concentrations of alcohol than those containing α2 (Mihic, 1999). Furthermore, alcohol concentrations lower than 100 mM are known to potentiate only α2β4, α4β4, α2β2 and α4β2 subtypes of neuronal nAChRs. In contrast, whereas α3β2 and α3β4 subtypes are not affected by those alcohol concentrations, α7 receptor function is inhibited (Harris, 1999). Higher alcohol concentrations are less selective and potentiate almost all nAChRs. As a result of the differential distribution of the aforementioned receptors, as well as of their subunits, throughout the brain (for example, 5-HT_3 and neuronal nAChRs are primarily expressed in the cerebral cortex and some limbic regions, whereas NR1/NR2B subtype of the NMDA receptor is primarily expressed in forebrain regions), alcohol affects some brain regions more than others.

Alcohol's direct modulation of the activity of ion channels or receptors can subsequently lead to a cascade of synaptic events involving multiple neurotransmitters. For instance, even though 5-HT_3 is considered to be an excitatory receptor, it is often expressed on inhibitory GABAergic interneurons, thus, activation of 5-HT_3 receptors by alcohol may contribute to some of the inhibitory actions of alcohol via increased release of GABA (Lovinger, 1999). Furthermore, activation of 5-HT_3 receptors is also known to increase release of dopamine (DA; 3,4-dihydroxyphenylethylamine) and glutamate (Lovinger, 1999). Similarly, neuronal nAChRs were found expressed on GABAergic and other interneurons and are known to modulate the release of DA, noradrenaline, GABA, glutamate and ACh itself (Narahashi, 2000; Moriguchi *et al*., 2007). Alcohol's direct inhibition of voltage-gated Ca^{2+} channels suppresses the release of several neurotransmitters. The resulting increased/decreased extracellular levels of different neurotransmitters might finally engage presynaptic autoreceptors, such as $GABA_B$ or metabotropic (for example, mGlu2/3) glutamate receptors, which in turn will reduce the effects of alcohol at these synapses.

In summary, despite the generally held view that alcohol is an unspecific pharmacological agent, recent molecular pharmacology studies demonstrate that alcohol does have a few primary targets, including NMDA, $GABA_A$, 5-HT_3 and nAChRs, 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. These *in vitro* findings are supported by drug (alcohol) discrimination studies. Thus, in numerous alcohol vs water discrimination experiments it has been shown that alcohol produces a stimulus complex composed of distinct components that are mediated by different receptor systems (Grant, 1999). In particular, an antagonism of NMDA receptors and an activation of $GABA_A$ receptors are involved in mediating the discriminative stimulus properties of alcohol. Furthermore, the alcohol stimulus effect is increased by N-cholinergic agonists and 5-HT_3 receptor agonists (Kostowski and Bienkowski, 1999). In conclusion, the acute psychotropic effects of alcohol are mediated by the complex interaction with these neurotransmitter/ion channel systems. Following this first hit of alcohol, a second wave of indirect effects on a variety of neurotransmitter/neuropeptide systems is initiated, leading finally to the typical behavioural effects of alcohol ranging from disinhibition to sedation and even hypnosis.

Initiation of alcohol consumption

The initiation of alcohol consumption strongly depends on the biological (genetically influenced) characteristics of an individual. One important variable is the level of response to an acute first alcohol challenge, that is, the initial sensitivity to alcohol. In other words, how fast does an individual become intoxicated, and at which concentrations does an alcohol intoxication stop signal fade in? That is, what are the subjective and objective responses associated with the consumption of a large amount of alcohol that lead to the cessation of further alcohol intake? The level of response to alcohol and the herewith associated intoxication stop signal are influenced by multiple genetic factors (Schuckit *et al*., 2004). Most importantly, however, differences in the activity of an individual's brain reinforcement system are thought to determine the reinforcing efficacy of alcohol.

The brain regions that play an important role in mediating the reinforcing effects of alcohol were identified by a variety of neuropharmacological studies, including lesion, microinjection and microdialysis experiments. Particularly, the extended amygdala and the mesolimbic DAergic pathway, including the ventral tegmental area (VTA), the nucleus accumbens (NAC) and the prefrontal cortex, are the main sites in the brain that mediate alcohol reinforcement. The mesolimbic DAergic pathway is known to be largely under the control of glutamatergic activity. Furthermore, the dorsal raphe nucleus 5-HT system modulates the DAergic activity of the VTA and the NAC. The VTA is also regulated by GABA and enkephalinergic projections from the ventral pallidum and the NAC, a cholinergic input from the pedunculopontine nucleus, and glutamatergic input from the prefrontal cortex. Finally, cannabinoids (CBs) and opioids can cause the release of DA in the NAC by activating DAergic neurons in the VTA. It is believed that enhanced DAergic activity acts as one signal that mediates alcohol reinforcement and highlights important stimuli such as alcohol-associated cues, and in so doing, functioning as a learning signal for the individual (Spanagel and Weiss, 1999).

Besides these biological and genetically determined responses (initial sensitivity, alcohol reinforcement) to an acute alcohol challenge in alcohol-naïve individuals, environmental influences, such as exposure to stress, have long been postulated to facilitate the onset of alcohol consumption. For example, the family, peers and society determine the level of exposure to stress and alcohol, whereas genes modulate how sensitive an individual responds to both. The resulting behaviours feed back to the social environment, modulating, and in the worst case increasing, further stress exposure (Zimmermann *et al*., 2007). Subsequently, alcohol drinking might be initiated to counteract some effects of stress (Pohorecky, 1981). It is thought that stressors can facilitate alcohol consumption by increasing the activity of

several neurobiological systems, such as the hypothalamic–pituitary–adrenal axis and extra-hypothalamic corticotropin-releasing factor (CRF) signalling (Liu and Weiss, 2002a). Mesolimbic DAergic neurons and GABAergic/serotonergic neurotransmission in the amygdala also integrate stress × alcohol interactions (Clarke *et al*., 2007; Heilig and Koob, 2007).

In conclusion, due to innate differences in several neurotransmitter and neuropeptide systems some individuals will have a weaker initial sensitivity to alcohol, and/or will find alcohol more reinforcing than others. These two mechanisms are viewed as crucial in the onset of alcohol consumption. Described in the following are the most important neurotransmitter and neuropeptide systems known to be involved in initial sensitivity and alcohol reinforcement. Initial sensitivity to high, intoxicating concentrations of alcohol in rats and mice can best be estimated by the duration of the loss of righting reflex (LORR), which is used as a measurement of CNS depression. High and low alcohol-sensitive lines of rats or mice have even been selectively bred based on the differences in their alcohol-induced LORR duration and have been used for pharmacological studies for many years. Alcohol reinforcement in rodents can best be measured by the conditioned place preference paradigm (Tzschentke, 2007) or the acquisition of voluntary alcohol consumption either under home cage drinking conditions or under operant conditions.

Glutamate

The effect of glutamate to alter alcohol-induced LORR was studied in mice. Mice were injected with a high, hypnotic dose of alcohol ($4\,g\,kg^{-1}$), which caused them to lose the righting reflex. After mice regained their righting reflex, they were immediately injected intracerebroventricularly with glutamate, which induced an instant return to the LORR within 60 s (Ferko, 1994). These data indicate that glutamate can enhance the central depressant action of alcohol. In line with this link between glutamate and LORR, it has been demonstrated that in healthy individuals with a family history of alcoholism, the response to the glutamate/NMDA receptor (NMDAR) antagonist ketamine was altered, as opposed to the response of those with no such family history (Petrakis *et al*., 2004). Similar findings were obtained in mice selectively bred for increased sensitivity to alcohol; these mice were more sensitive to ketamine treatment (Meyer and Phillips, 2003), suggesting that altered NMDAR function contributes to the altered response to an acute alcohol challenge. This finding, however, is not surprising, given the fact that the NMDAR is a primary target of alcohol's action.

NMDAR antagonists are capable of preventing the acquisition of alcohol-induced conditioned place preference in rats (Biala and Kotlinska, 1999). Furthermore, alcohol self-administration in a free-choice operant task (water vs 10% alcohol) was attenuated by microinjection into the NAC of 2-amino-5-phosphopentanoic acid—a competitive NMDAR antagonist (Rassnick *et al*., 1992). These results suggest that glutamate neurotransmission in the NAC may modulate alcohol self-administration and its reinforcing effects.

The glutamatergic system in turn is tightly linked to the nitric oxide (NO) pathway. Thus, stimulation of NMDARs leads to a calcium influx, and the binding of calcium to calmodulin activates neuronal NO synthase, which produces NO. The close link between the glutamatergic/NMDA receptor system and NO production implies that the neuronal NO synthase gene is also involved in the modulation of acute effects of alcohol. In fact, several pharmacological studies and studies using neuronal NO synthase knockout mice show that NO signalling also modulates the duration of LORR induced by alcohol and alcohol reinforcement (Spanagel *et al*., 2002).

GABA

Highly alcohol-sensitive and less alcohol-sensitive rat lines have marked line differences in GABA-mediated events, and these are correlated with the sedative effects of alcohol. Using different $GABA_A$ receptor subunit knockout mouse models for the measurement of alcohol sensitivity provides the currently strongest evidence for a role of α1 subunits in alcohol-induced LORR (Boehm *et al*., 2006). Polymorphisms in $GABA_A$ receptor subunits have also been linked to alcohol response in humans (Dick *et al*., 2006).

Interestingly, Sardinian alcohol non-preferring rats, selected for their low alcohol preference and consumption, carry a point mutation (*R100Q*) in the gene coding for the $GABA_A$ receptor α6 subunit. Rats carrying this mutation voluntarily drank less alcohol than did normal rats during the acquisition phase, but this difference was lost during the maintenance phase (Sanna *et al*., 2004). However, in a recent study by Botta *et al*. (2007) it has been demonstrated that the α6-*R100Q* $GABA_A$ receptor subunit polymorphism did not change the acute alcohol sensitivity of extrasynaptic receptors. Furthermore, α6 subunit knockout mice did not differ in the sensitivity to the hypnotic effects of alcohol as measured by LORR (Homanics *et al*., 1997). Activation of the $GABA_B$ receptor was shown to suppress acquisition of alcohol-drinking behaviour in rats (Colombo *et al*., 2002a; Carai *et al*., 2005).

nAChR/glycine

It has been shown that alcohol-induced stimulation of the mesolimbic DA system involves central nACh as well as strychnine-sensitive glycine receptors (Ericson *et al*., 1998; Söderpalm *et al*., 2000; Molander *et al*., 2005), which suggests the possible involvement of these receptors in the initiation phase of alcohol consumption. Thus, alcohol-induced DA overflow within the NAC is mediated by nAChR located in the VTA as mecamylamine infused into this area can antagonize this effect and can reduce voluntary alcohol drinking (Ericson *et al*., 1998, 2003).

DA/5-HT

Both neurotransmitters play, if at all, only a minor role in mediating sensitivity to high doses of alcohol. Thus, various manipulations of these systems did not produce consistent alterations in LORR measurements. In contrast, both systems

are crucial for the initiation of alcohol reinforcement. Following the key publication by Gessa *et al*. (1985), which showed that low doses of alcohol produce a dose-dependent increase in the firing rate of DAergic neurons in the VTA, it was later consistently shown that alcohol stimulates DA transmission in the mesolimbic pathway (Di Chiara and Imperato, 1988). With respect to 5-HT, it was shown that alcohol potentiates the action of this neurotransmitter via the 5-HT_3 receptor (Lovinger and Zhou, 1994), and it is suggested that inborn DAergic and/or 5-HTergic dysfunction might be of importance for the initial alcohol preference. For instance, low levels of DA and 5-HT were seen in different alcohol-preferring P, high-alcohol-drinking (HAD) and alcohol-preferring Fawn-Hooded rat lines as compared to their alcohol non-preferring counterpart lines (McBride and Li, 1998). In agreement, 5-HT_3 receptor antagonists were shown to suppress the acquisition of voluntary alcohol consumption in alcohol-preferring P rats (Rodd-Henricks *et al*., 2000).

Cannabinoids

There is only little evidence that CBs influence the initial sensitivity to hypnotic doses of alcohol. However, alcohol reinforcement processes are modulated by this system, and it is suggested that alcohol reward is 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 (Hungund and Basavarajappa, 2000). Genetically selected alcohol-preferring msP or alcohol-preferring AA (Alko alcohol) rat lines show specific differences in the organization of the brain endocannabinoid system in a number of brain regions when compared with unselected Wistars or alcohol-avoiding Alko non-alcohol (ANA) rat lines (Cippitelli *et al*., 2005; Hansson *et al*., 2007) and CB1 receptor antagonism has been reported to specifically suppress acquisition of alcohol-drinking behaviour in rodents (Colombo *et al*., 2005). More importantly, CB1 receptor knockout mice display reduced alcohol-induced conditioned place preference and self-administration (Wang *et al*., 2003; Thanos *et al*., 2005a). Furthermore, the study from Wang *et al*. (2003) showed that there was an age-dependent decline in ethanol preference and intake in the wild-type but not CB1 knockout mice, which is consistent with the idea that reward-dependent mechanisms become less important with age and that a decrease in activity within the endogenous CB system might correlate with these events. Furthermore, the human CB1 receptor, which is encoded by the CNR1 gene, may play a role in the development of alcoholism as it has recently been reported by Zuo *et al*. (2007) in a large case–control sample.

Opioids

Endogenous opioids are crucial for mediating alcohol-induced reward. The opioid receptor antagonist naltrexone reverses alcohol-induced DA release in the NAC in rats, and suppression of operant alcohol-reinforced behaviour by naltrexone is associated with attenuation of the alcohol-induced increase in dialysate DA levels in the NAC (Gonzales and Weiss, 1998). 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 (Herz, 1997). Furthermore, alcohol-preferring AA rats show lower opioidergic activity in areas involved in alcohol reinforcement (Nylander *et al*., 1994), and many other studies have also reported innate differences in opioid systems in other alcohol-preferring and -avoiding lines of animals, such as AA vs ANA, and P vs alcohol non-preferring rats, or C57BL/6 vs DBA/2 mice (Herz, 1997; Jamensky and Gianoulakis, 1997; McBride and Li, 1998). In line with the conclusion that endogenous opioid systems are crucial in mediating alcohol reward, naltrexone was shown to slow down the acquisition of alcohol drinking in mice (Phillips *et al*., 1997).

CRF/NPY

Innate hyperactivity of the CRF system and hypoactivity of the neuropeptide Y (NPY) system are known to promote alcohol consumption. Thus, high-alcohol-preferring msP rats show an anxious-like phenotype and higher density of CRF1 receptor in several limbic areas related to alcohol drinking motivation (Heilig and Koob, 2007). Thiele *et al*. (1998) demonstrated that NPY-deficient mice show increased alcohol consumption compared with wild-type mice, and are also less sensitive to the hypnotic effects of alcohol, as shown by their more rapid recovery from LORR. In contrast, transgenic mice that overexpress an NPY gene in neurons have a lower preference for alcohol and are more sensitive than controls to the hypnotic effects of this drug. These data are direct evidence that alcohol consumption and resistance are inversely related to NPY levels in the brain (Thiele *et al*., 1998). The alcohol-preferring P rats and non-selected C57BL/6J mice show low constitutive levels of NPY, which has been suggested to contribute to their known high level of alcohol consumption (Hwang *et al*., 2004; Hayes *et al*., 2005). In summary, transgenic work with NPY and null-mutant mice, as well as studies with alcohol-preferring animals, has implicated NPY in the control of alcohol consumption, suggesting that genetic variations of the NPY gene may also contribute to the heritability of alcoholism. In fact, in a recent study an association of specific variants of the NPY gene and the onset of alcoholism has been shown (Mottagui-Tabar *et al*., 2005). The same applies for the CRF system, as a link between the stress system and vulnerability to alcohol drinking has been recently documented in humans—polymorphisms of the genes encoding CRF1 receptor have been associated with alcohol consumption (Treutlein *et al*., 2006).

In summary, the inborn differences in the activity of several neurotransmitter systems are one important reason why some individuals initiate alcohol drinking and others do not. This might as well explain the initiation of alcohol use early in life. The presence/absence of one or several alternative alleles of particular genes therefore results in an alcohol-sensitive phenotype that in the appropriate environment may lead to the initiation of alcohol drinking because the response of these individuals to appetitive properties of this drug will be much greater.

Maintenance of alcohol consumption

Once an alcohol drinking behaviour is established, further chronic alcohol consumption influences brain function by altering the balance between inhibitory and excitatory neurotransmission through different neurotransmitter and neuropeptide systems. GABA and glutamate are probably the most studied neurotransmitter systems in this respect. Thus, short-term alcohol consumption increases $GABA_A$ receptor function, while prolonged drinking due to counteradaptative processes has the opposite effect. This decrease in $GABA_A$ function may result from a decreased number of receptors or from changes in the subunit composition of this receptor, leading to a decreased receptor sensitivity regarding neurotransmission (Mihic, 1999). In contrast, glutamatergic activity is decreased by an acute exposure to alcohol, mostly due to its antagonistic effect at the NMDA receptor; hence, following chronic alcohol intake glutamate receptors appear to adapt to the inhibitory effects of alcohol by increasing their excitatory activity (Hoffman *et al*., 1990). In addition to the action on GABA and glutamate, chronic alcohol consumption affects several other neurotransmitter systems as well, such as the mesolimbic DAergic system, the opioidergic system and the endocannabinoid system at the level of the NAC, VTA and amygdala, as well as the brain stress CRF and antistress NPY systems; although interactions between alcohol and these transmitter systems seem to be more complex (Heilig and Koob, 2007).

The effect of chronic alcohol consumption on multiple neurotransmitter systems that underlie the vulnerability to excessive alcohol drinking behaviour is supported by substantial evidence from preclinical research. Most evidence stems from a great number of alcohol drinking studies in rodents. In particular, a number of neurochemical differences were found in genetically selected alcohol-preferring rat lines as compared with non-preferring lines. The most commonly used lines are the Finnish alcohol-preferring (AA), the Sardinian-preferring (SP), the Indiana University alcohol-preferring (P) and the HAD rat lines (Bell *et al*., 2006; Ciccocioppo *et al*., 2006; Overstreet *et al*., 2006; Sommer *et al*., 2006). As an example, low levels of DA and 5-HT in several limbic structures were seen in alcohol-preferring P and HAD rat lines in comparison to their alcohol non-preferring counterparts. Furthermore, there are several differences between the GABAergic, CB, CRF and NPY systems in alcohol-preferring rats and those in non-preferring animals (McBride and Li, 1998; Heilig and Thiele, 2005; Hansson *et al*., 2007). Thus, the data from alcohol-preferring animals confirm the involvement of multiple neurotransmitter systems in the maintenance of high/excessive alcohol consumption.

In addition, genetically modified animals, especially knockout mouse models, are very helpful in identifying genes and neurochemical substrates that are involved in mediating the effects of chronic alcohol intake (Crabbe *et al*., 2006). In summary, the combination of gene deletion studies and pharmacological experiments in alcohol-preferring rat lines has produced a consistent set of results, and has provided a picture of the causes underlying high chronic alcohol consumption, as will be specifically shown in the next sections.

Glutamate

Adaptive responses such as changes in the number and/or affinity of synaptic glutamate receptors or their subunits will occur to counterbalance the acute inhibitory effect of alcohol on NMDAR function and glutamate release. However, the outcome of pharmacological studies using NMDAR antagonists with regard to alcohol self-administration is inconclusive, showing that different NMDAR antagonists can reduce or have no effect on alcohol intake (Shelton and Balster, 1997; Bienkowski *et al*., 1999). In addition, NR2A subunit deletion in mice does not affect voluntary alcohol intake (Boyce-Rustay and Holmes, 2006).

The application of the AMPA (DL-α-amino-3-hydroxy-5-methylsoxazole-4-propionate)/kainate receptor antagonist GYKI 52468 did not alter operant responding for alcohol (Stephens and Brown, 1999). Experiments with knockout mice also do not suggest the involvement of these receptors in the maintenance of alcohol drinking, as both GluR1 and GluR3 deletions did not have any effect on either home cage alcohol drinking or operant self-administration (Cowen *et al*., 2003; Sanchis-Segura *et al*., 2006).

Similarly, no changes in operant alcohol self-administration by alcohol-preferring P rats were found by use of a metabotropic glutamate receptor1 (mGluR1) antagonist (Schroeder *et al*., 2005a). Neither agonists nor antagonists acting at mGluR2/3 receptors had an effect on maintenance responding for alcohol under operant conditions in P rats (Schroeder *et al*., 2005a; Rodd *et al*., 2006). No difference in alcohol consumption was seen in mGluR4 knockout mice (Blednov *et al*., 2004). In contrast, mGluR5 antagonists were capable of reducing alcohol-reinforced responding in mice (Hodge *et al*., 2006) and alcohol-preferring P and alcohol-preferring Fawn-Hooded rats (Cowen *et al*., 2005; Schroeder *et al*., 2005a).

GABA

Chronic alcohol consumption is associated with a decrease in the brain $GABA_A$ receptor density and brain region-specific up- or downregulation of α and β subunit gene expression (Golovko *et al*., 2002). Knockout mice lacking different $GABA_A$ receptor subunits were examined in several alcohol-related paradigms and it was shown that α1, α2, α5 and δ subunit deletion leads to lower alcohol consumption (Mihalek *et al*., 2001; Boehm *et al*., 2004; Crabbe *et al*., 2006; June *et al*., 2007). The critical determinant of alcohol's actions on $GABA_A$ receptors seems to be protein kinase C (PKC) activity. Thus, mice lacking PKCε showed increased sensitivity of $GABA_A$ receptors to allosteric modulation by alcohol (Hodge *et al*., 1999). In contrast, $GABA_A$ receptors are less sensitive to alcohol's effects in mice lacking PKCγ (Harris *et al*., 1995). Accordingly, PKCε knockout mice are known to consume less alcohol (Hodge *et al*., 1999), whereas PKCγ knockouts show increased voluntary alcohol consumption (Bowers and Wehner, 2001).

Pharmacological manipulations of $GABA_A$ receptors were studied in alcohol-preferring rat lines. Thus, negative allosteric modulators of the $GABA_A$ receptor were shown to reduce alcohol consumption in several alcohol-preferring rat lines (Rassnick *et al*., 1993a; Wegelius *et al*., 1994). Additionally,

antagonism of $GABA_A$ receptors within the VTA (Nowak *et al*., 1998) or increasing the activity of those receptors in NAC (June *et al*., 1998) suppressed alcohol consumption in alcohol-preferring P rats, suggesting the particular importance of these nuclei in the maintenance of alcohol drinking.

Metabotropic $GABA_B$ receptors might also play a crucial role in controlling the levels of chronic alcohol intake. Thus, the $GABA_B$ agonist baclofen suppressed voluntary alcohol consumption in alcohol-preferring sP rats. However, repeated use of baclofen might lead to the development of tolerance (Colombo *et al*., 2000; Carai *et al*., 2005). Interestingly, co-administration of a positive allosteric modulator of the metabotropic $GABA_B$ receptor such as CGP7930 (3-(3′,5′-di-tert-butyl-4′-hydroxy)phenyl-2,2-dimethylpropanol) enhances baclofen's potency and reduces the development of tolerance (Adams and Lawrence, 2007).

nAChR/glycine

Cholinesterase inhibitors were shown to reduce alcohol intake and preference in alcohol-preferring AA rats (Doetkotte *et al*., 2005). It remains, however, to be established what subunit composition of the nAChRs is most important in this respect. To date, it is known that α4β2 and α7 subtypes of nAChRs are not playing an important role in alcohol consumption (Lê *et al*., 2000; Ericson *et al*., 2003), whereas antagonism of α3β2 and β3 subunits of the nAChR were capable of reducing voluntary alcohol consumption in both rats and mice (Larsson *et al*., 2004; Jerlhag *et al*., 2006b).

Modulation of the activity of the glycinergic system might lead to reduced alcohol self-administration. Thus, Molander *et al*. (2007) recently showed that the glycine reuptake inhibitor Org25935—acting specifically on the glycine transporter 1 (GlyT1)—decreases alcohol intake and preference in male Wistar rats by increasing extracellular glycine levels that primarily activate inhibitory strychnine-sensitive glycine receptors.

Channels

Katsura *et al*. (2006) demonstrated an increased expression of the α1C, α1D and α2/δ1 subunits of L-type voltage-sensitive calcium channels in the mouse cerebral cortex following chronic alcohol exposure. Accordingly, the Ca^{2+} channel antagonist verapamil was able to reduce alcohol intake in alcohol consuming monkeys (Rezvani *et al*., 1991). Knockout mice models have shown that both N-type Ca^{2+} and GIRK2 channels might also have a certain role in the maintenance of alcohol consumption (Blednov *et al*., 2001; Newton *et al*., 2004). Reduced alcohol consumption in humans was achieved by use of anticonvulsants, which are known to exert their effects via multiple mechanisms of action; however, one common feature of these compounds is the inhibition of voltage-gated Na^+ and Ca^{2+} channels (Johnson, 2004; Johnson *et al*., 2004).

DA/5-HT

Despite the fact that alcohol-induced DA release within the NAC is critically involved in the initiation of alcohol reinforcement processes (Di Chiara and Imperato, 1988; Spanagel and Weiss, 1999), 6-hydroxy DA-induced lesion of the mesolimbic tract failed to alter voluntary self-administration in rats, suggesting a less important role of DA in maintaining alcohol consumption (Rassnick *et al*., 1993b). However, postsynaptic changes in DA receptor signalling seem to be involved in the maintenance of voluntary alcohol intake as DA D1 and D2 receptor knockout mice show alterations in alcohol consumption (Crabbe *et al*., 2006) and D1, D2 and D3 receptor agonists and antagonists were capable of modulating alcohol consumption in common stock rats (Pfeffer and Samson, 1988; Russell *et al*., 1996; Cohen *et al*., 1998) and alcohol-preferring rats as well (Dyr *et al*., 1993; McBride and Li, 1998; Thanos *et al*., 2005b).

Knockout mouse models and pharmacological manipulations of various components of the 5-HT system indicate a modulatory role of 5-HT in voluntary alcohol consumption. Thus, deletion of 5-HT transporters (Kelaï *et al*., 2003) or overexpression of $5\text{-}HT_3$ receptors (Engel *et al*., 1998) leads to a reduction in alcohol self-administration as compared with control mice. Deletion of $5\text{-}HT_{1B}$ receptors was shown to increase alcohol intake (Crabbe *et al*., 1996; but see also Crabbe *et al*., 2006). Pharmacological manipulations of 5-HT system activity revealed that administration of serotonin reuptake inhibitors, $5\text{-}HT_1$, $5\text{-}HT_2$ and $5\text{-}HT_3$ agonists as well as antagonists were capable of reducing alcohol consumption in common stock as well as alcohol-preferring animals (Zhou *et al*., 1998; Lê and Funk, 2005; Ciccocioppo *et al*., 2006; Overstreet *et al*., 2006).

Cannabinoids

Endocannabinoid transmission is acutely inhibited by alcohol (Ferrer *et al*., 2007) and thus, becomes hyperactive during chronic alcohol administration, as revealed by the increase in the levels of endocannabinoids and the down-regulation of CB1 receptors (Bilbao and Rodriguez de Fonseca, 2008). Thus, chronic alcohol administration was found to be associated with an increased formation of the endocannabinoids anandamide and 2-AG (Basavarajappa and Hungund, 1999; Basavarajappa *et al*., 2000). Most of the behavioural studies have focused on the pharmacological manipulation of the CB1 receptor showing that agonists increase (Colombo *et al*., 2002b), whereas antagonists decrease alcohol intake and preference (Arnone *et al*., 1997; Gessa *et al*., 2005). In agreement, CB1 receptor knockout mice were shown to consume less alcohol in most of the studies (Crabbe *et al*., 2006).

Opioids

Opioid μ and κ receptor knockout mice were shown to exhibit lower alcohol self-administration, suggesting the significance of these receptors in the maintenance of alcohol consumption (Roberts *et al*., 2000; Kovacs *et al*., 2005), whereas δ knockouts displayed greater alcohol preference and intake (Roberts *et al*., 2001). In accordance, selective antagonists acting at either μ or κ opioid receptors were able to reduce alcohol consumption, which supports the hypothesis that following chronic alcohol intake the endorphin/μ

opioid receptor system as well as the dynorphin/κ opioid receptor system is dysregulated (McBride and Li, 1998; Walker and Koob, 2008).

CRF/NPY

A number of pharmacological studies using antagonists for the CRF1 receptor have demonstrated that activity of this receptor is responsible for excessive alcohol self-administration in non-selected rodents (Chu *et al*., 2007; Funk *et al*., 2007) as well as in high-alcohol-preferring msP rats (Hansson *et al*., 2006; Gehlert *et al*., 2007). In line with these findings, CRF1 receptor-deficient mice do not show any increase in self-administration following an induction of dependence (Chu *et al*., 2007). It is, however, important to emphasize that CRF1 receptor antagonism is only efficient in animal models with excessive alcohol consumption but has only little effect on the maintenance of moderate voluntary alcohol consumption in the home cage. A possible reason is that long-term upregulation of CRF1 receptors is observed in the amygdala only following chronic and excessive alcohol drinking (Heilig and Koob, 2007; Sommer *et al*., 2008). In this context, George Koob and Le Moal propose that changes in the activity of CRF system maintain hedonic stability in an allostatic state, as opposed to a homoeostatic state (Koob, 2003; Koob and Le Moal, 2006) and thereby contribute to excessive alcohol consumption.

The NPY system is also believed to be involved in the maintenance of alcohol self-administration as administration of NPY antagonists have been shown to decrease alcohol self-administration in both genetically selected and non-selected alcohol-preferring lines (Gilpin *et al*., 2003; Schroeder *et al*., 2005b) as well as in animals with a history of dependence (Rimondini *et al*., 2005).

In summary, continuous alcohol use affects a wide range of brain neurotransmitter/neuromodulator systems, leading to adaptive, long-lasting changes. Thus, it is probably not possible to determine which of these systems contributes the most to the development of alcohol dependence, as it is likely to be interplay between different brain systems that is responsible for such a pathological process, rather than a specific action of alcohol exclusively on one of the systems. Some of those changes, however, cause the transition between controlled and compulsive alcohol use. This occurs only in a limited proportion of about 10–15% of alcohol users and is the result of initial genetic differences between individuals and environmental factors at critical phases of the life course.

Craving and reinstatement of alcohol seeking

There are opposing views among experts working in the field of alcoholism and drug abuse research regarding the term 'craving' as to whether it describes a physiological, subjective or behavioural state, if it is necessary at all to explain addictive behaviour, or whether it is an epiphenomenon that is not necessary for the production of continued alcohol use in alcohol-dependent patients (for a controversial discussion on craving, see Spanagel and Hölter (2000). An expert committee gathered by the United Nations International Drug Control Programme and the World Health Organization agreed on the definition of craving as 'the desire to experience the effect(s) of a previously experienced psychoactive substance'. Craving can occur even after long-term abstinence and is typically provoked by stress, conditioned alcohol-associated stimuli and internal stimuli such as changes in mood state. It is believed that residual alterations in several neurotransmitter systems that cause functional deficits in reward-related pathways and sensitization to environmental stimuli that predict drug effects are responsible for alcohol craving. Thus, like other drugs of abuse, alcohol sensitizes neural pathways important in signalling incentive motivation and thereby drug-paired stimuli become conditioned incentives that activate a central motivational state (Tiffany and Conklin, 2000; Addolorato *et al*., 2005b). Alternatively, it is suggested that drug-paired stimuli elicit conditioned-withdrawal effects, which mimic the autonomic components of withdrawal, and thereby may also induce alcohol craving (Littleton, 2000). These two different mechanisms are best captured by the terms reward and relief craving (Heinz *et al*., 2003). In fact, among alcohol-dependent patients, it is possible to distinguish between reward and relief cravers (Project PREDICT; Karl Mann, personal communication), and this might have important implications for the treatment of craving, as it is suggested that reward craving might result from DAergic/opioidergic dysregulation, whereas relief craving might result from either GABAergic/glutamatergic dysregulation or dysregulation in the CRF signalling pathway (Verheul *et al*., 1999; Heilig and Koob, 2007).

Markou *et al*. (1993) conceptualized craving within the framework of incentive motivational theories of behaviour and modified the definition of craving as the 'incentive motivation to self-administer a psychoactive substance'. Such an operational definition of craving has the advantage of making the phenomenon of craving accessible to experimental investigation and making it measurable. On the basis of this definition, animal models of alcohol craving have been developed. In particular, the measurement of a drug-seeking component in rats and mice can be established. The procedure applied to study alcohol craving and seeking in animals is the so-called reinstatement model (Shaham *et al*., 2003; Sanchis-Segura and Spanagel, 2006). In this procedure, an animal is trained to self-administer alcohol and is then subjected to extinction—that is, the animal is tested under conditions of non-reinforcement until operant responding appears to be extinguished. When the animal reaches some criterion of unresponsiveness, various stimuli are presented. A stimulus is said to reinstate the alcohol-seeking behaviour if it causes renewed responding, that is, lever pressing, without any further response-contingent drug reward. Reinstatement of alcohol seeking can be used to study the neurobiological and molecular basis of craving as there appears to be a good correspondence between the events that induce alcohol seeking in laboratory animals and those that provoke craving in humans. At least four events can reinstate responding: (i) alcohol priming—that is the injection of a small dose of alcohol, (ii) stress, (iii) nicotine and

(iv) conditioned stimuli. The data derived from studies using the reinstatement model suggest that the neuronal substrates that mediate priming-, stress- and cue-induced reinstatement are not identical (Shaham *et al*., 2003; Sanchis-Segura and Spanagel, 2006) and therefore imply the involvement of different neurobiological pathways in provoking craving, which is in line with the concept of reward and relief craving (Verheul *et al*., 1999). Importantly, the reinstatement model already has a certain degree of pharmacological validation. Thus, acamprosate and naltrexone are known to reduce craving and relapse in alcoholic patients and can also reduce or even block cue-induced reinstatement of alcohol-seeking behaviour in rodents (Katner and Weiss, 1999; Bachteler *et al*., 2005). The reinstatement paradigm is now a well-established model in rats, and it is only recently that it has become possible to transfer this model to mice (Sanchis-Segura *et al*., 2006; Heidbreder *et al*., 2007; Zghoul *et al*., 2007). In the future, this paradigm will be frequently used to study genetically modified mice to precisely pin down the genes and brain sites involved in alcohol-seeking behaviour.

Described in the following are the different neurochemical substrates that have been implicated in the reinstatement of alcohol-seeking behaviour in animals and craving in humans.

Glutamate

Adaptive changes of glutamatergic systems may cause hyperexcitability of the CNS during withdrawal or conditioned withdrawal and further possibly represent one mechanism causing alcohol craving (Littleton, 2000; Gass and Olive, 2008). However, NMDA receptors seem to play a minor role in conditioned alcohol-seeking responses (Bäckström and Hyytia, 2004; Bachteler *et al*., 2005), whereas non-NMDA ionotropic glutamate receptors, especially AMPA receptors, are involved in this behaviour (Bäckström and Hyytia, 2004; Sanchis-Segura *et al*., 2006). The use of AMPA receptor subunit GluRC knockout mice revealed an exceptional role of this subunit in cue-induced alcohol-seeking behaviour (Sanchis-Segura *et al*., 2006). Not only ionotropic AMPA receptors but also mGluRs might be involved in alcohol-seeking behaviour. In particular, mGluR5 receptor antagonists are effective in the reinstatement model (Bäckström *et al*., 2004). Activation of group II mGluRs attenuates both stress- and cue-induced reinstatement behaviour (Zhao *et al*., 2006). However, another study using agonists acting at mGluR2/3 and mGluR8 receptors showed that significant effects of treatment on cue-induced reinstatement occurred only at doses that also decreased spontaneous locomotor activity (Bäckström and Hyytiä, 2005).

GABA

Priming injections of the $GABA_A$ receptor-positive modulator allopregnanolone dose-dependently reinstated extinguished responding for alcohol (Nie and Janak, 2003), suggesting GABAergic control over alcohol seeking. The $GABA_A$ receptor agonist muscimol administered directly into median raphe nucleus was also able to reinstate alcohol seeking (Lê *et al*., 2008), implying an important role of this nucleus in this behaviour. Baclofen, an agonist acting at the $GABA_B$ receptor, was shown to reduce the motivation to consume alcohol in rats (Colombo *et al*., 2003b), suggesting a possible role of this receptor in alcohol craving as well. There are also indications that this drug might be efficient in reducing alcohol craving in humans (Addolorato *et al*., 2002, 2005a).

nAChR

Priming injections of nicotine reinstate alcohol seeking after a drug-free period in rats (Lê *et al*., 2003, 2006a), demonstrating an additional event that can induce craving. In fact, alcohol seeking and nicotine seeking are tightly associated most likely through their common action on nAChR. What is more, nAChR in the VTA mediate the DA-activating and -reinforcing properties of alcohol cues (Löf *et al*., 2007).

Channels

The anticonvulsant drug lamotrigine, which was shown to inhibit Na^+ channel activity and in turn reduce glutamate release, was found to significantly decrease cue-induced reinstatement of alcohol-seeking behaviour in rats (Vengeliene *et al*., 2007).

DA/5-HT

D1, D2 and D3 DA receptor antagonists were shown to reduce cue-induced reinstatement of alcohol-seeking behaviour (Liu and Weiss, 2002b; Vengeliene *et al*., 2006). The exposure of abstinent individuals to environmental cues previously associated with alcohol is known to induce mesolimbic DA release (Löf *et al*., 2007), suggesting an important role of augmented extracellular DA levels in cue-induced alcohol craving/seeking.

Administration of a $5\text{-}HT_{1A}$ agonist, known to decrease 5-HT release, into median raphe nucleus reinstated alcohol seeking (Lê and Funk, 2005), whereas systemic injections of 5-HT uptake blockers as well as $5\text{-}HT_3$ antagonists attenuated intermittent foot-shock stress-induced reinstatement of alcohol seeking in rats (Lê *et al*., 1999, 2006b; Lê and Funk, 2005).

Cannabinoids

Acute administration of an anandamide reuptake inhibitor AM404 was incapable of altering cue-induced reinstatement of alcohol-seeking behaviour (Cippitelli *et al*., 2007). In contrast, acute administration of a CB1 receptor antagonist has been shown to suppress this behaviour (Cippitelli *et al*., 2005), suggesting a possible role of this particular receptor in cue-elicited craving for alcohol.

Opioids

Unselective opioid receptor antagonists such as naltrexone are efficient in reducing cue-induced responding of alcohol seeking (Katner *et al*., 1999; Hyytiä, 2005) as well as priming-induced alcohol-seeking behaviour (Lê *et al*., 1999).

However, stress-induced reinstatement is not affected by this treatment (Lê *et al*., 1999). Furthermore, selective antagonism of μ or δ opioid receptors inhibited alcohol-seeking behaviour elicited by drug-related environmental stimuli (Ciccocioppo *et al*., 2002). A recent positron emission tomography study is in line with these preclinical findings as by means of the selective μ opioid receptor ligand carfentanil, a correlation of craving and μ opioid receptor availability in alcohol-dependent subjects could be detected (Heinz *et al*., 2005).

CRF

Cue- and stress-induced reinstatement has been found to be blocked by CRF1 receptor antagonists (Lê *et al*., 2000; Gehlert *et al*., 2007; Marinelli *et al*., 2007). Administration of a CRF1 receptor antagonist into median raphe nucleus blocked stress-induced reinstatement (Lê *et al*., 2002), suggesting an interaction between CRF and 5-HT systems within this nucleus. In addition, the CRF system within the central nucleus of the amygdala is also believed to mediate stress-induced reinstatement of alcohol-seeking behaviour (Funk *et al*., 2003). Antagonism of the CRF1 receptor was also shown to reduce reinstatement of alcohol seeking induced by the α2-adrenoreceptor antagonist yohimbine (Marinelli *et al*., 2007).

In summary, exposure to environmental cues or stressors activates the mesolimbic DAergic system, which, together with a specific memory for alcohol, will induce an alcohol-seeking response. However, the fact that reinstatement of alcohol seeking can be initiated by at least four different events namely conditioned cues, priming, stress and nicotine shows that neurochemically and nueroanatomically distinct pathways can induce such a response, which is in accordance with the multi-pathway model of alcohol craving proposed by Verheul *et al*. (1999). Given these findings, it is very unlikely that a specific pharmacological intervention will lead to overall blunted craving responses in alcohol-dependent patients.

Relapse to alcohol use

The major concern in the clinical work with alcohol-dependent patients is the prevention of relapse during periods of abstinence. In fact, relapses to alcohol use are known to occur in the majority of clinical cases if no appropriate treatment is provided, indicating that they are a fundamental part of this disease and should be considered as one of the central features of an addicted behaviour.

Animals that had long-term voluntary access to alcohol and became deprived for several days up to months are known to robustly but transiently increase their alcohol intake over baseline drinking following the re-presentation of alcohol (Le Magnen, 1960; Sinclair and Senter, 1967). This robust phenomenon is called the alcohol deprivation effect (ADE) and is observed across several species, including rats, mice and monkeys (Sinclair and Senter, 1967; Sinclair, 1971; Salimov and Salimova, 1993). The mechanisms underlying the temporary increase in alcohol consumption following periods of abstinence are not clear, although it might be caused by an increase in the reinforcing value of alcohol; alternatively, alcohol itself might be acting as a cue (that is, smell, taste) or as a priming stimulus. Naltrexone and acamprosate, two medications currently used in clinical practise to treat alcohol-dependent patients, were shown to effectively reduce an ADE in rodents (Spanagel *et al*., 1996; Hölter and Spanagel, 1999). This pharmacological validation of the ADE model provides some predictive validity in the search for new antirelapse compounds by the use of this animal model. In fact, in recent years the ADE model has become a widely used paradigm in examining the efficacy of new potential pharmacological agents to prevent relapse drinking (Spanagel and Zieglgänsberger, 1997; Heyser *et al*., 1998; Rodd-Henricks *et al*., 2000), and several neurochemical substrates that mediate the ADE have been identified—these studies are summarized in the following paragraphs.

Glutamate

One major hypothesis in the field of alcohol research proposes that the glutamatergic system is critically involved in addictive behaviour especially in relapse (Tsai and Coyle, 1998; Gass and Olive, 2008). The clinically used drug acamprosate, known to reduce a hyper-glutamatergic system (Spanagel *et al*., 2005), was capable of reducing the ADE in Wistar rats under home cage and operant conditions (Spanagel *et al*., 1996; Hölter *et al*., 1997; Heyser *et al*., 1998). Furthermore, the study by Hölter *et al*. (2000a) has demonstrated that chronic treatment with a non-competitive NMDA channel antagonist selectively abolished the increased alcohol intake during the ADE. Similarly, reduction of relapse-like alcohol drinking after a deprivation phase occurred after administration of competitive and non-competitive antagonists acting at specific binding sites of the NMDA receptor (Vengeliene *et al*., 2005). Reduced relapse-like drinking was also shown with use of an AMPA/kainate antagonist, whereas AMPA subunit deletion studies suggest a crucial role of the GluRC subunit in the expression of ADE (Cowen *et al*., 2003; Sanchis-Segura *et al*., 2006). Antagonism of mGluR5 was also capable of reducing relapse-like alcohol self-administration in Wistar rats (Bäckström *et al*., 2004) as well as of reducing repeated ADEs in alcohol-preferring P rats (Schroeder *et al*., 2005a). And finally, agonists acting at mGluR2/3 receptors were found to be effective in reducing relapse-like alcohol self-administration (Rodd *et al*., 2006). In conclusion, a decrease in glutamatergic activity and signalling in general seems to be sufficient to reduce relapse behaviour.

GABA

Administration of both the $GABA_A$ agonist diazepam and antagonist flumazenil decreased post-deprivation alcohol consumption, whereas the inhibition of the GABA transporter by tiagabine resulted in an increase of the ADE (Schmitt *et al*., 2002). $GABA_B$ agonists were shown to decrease the expression of an ADE in alcohol-preferring sP rats (Colombo *et al*., 2003a). In general, these findings

suggest that the GABAergic system is involved in the expression of relapse-like drinking behaviour.

nAChR/glycine

A recent clinical study by Mann *et al*. (2006) showed that relapsed patients treated with the cholinesterase inhibitor galantamine consumed less alcohol per drinking day than patients treated with placebo.

The glycine reuptake inhibitor Org25935 significantly reduced alcohol intake after introduction of an alcohol deprivation period in rats (Molander *et al*., 2007).

Channels

Administration of the anticonvulsant drug lamotrigine completely abolished the expression of an ADE in Wistar rats (Vengeliene *et al*., 2007).

DA/5-HT

Free-choice alcohol drinking by alcohol-preferring P rats alters DA and 5-HT neurotransmission in the NAC during a deprivation phase. In the absence of alcohol, extracellular DA levels were enhanced for at least 2 weeks, suggesting that changes in monoaminergic systems may be in part responsible for increased alcohol drinking observed during the ADE (Thielen *et al*., 2004).

Following long-term alcohol consumption with repeated deprivation phases, alcohol-preferring iP rats exhibited increased binding sites for D1 and D2 receptors in NAC, dorsal striatum and subamygdala regions (Sari *et al*., 2006). Furthermore, an upregulation of DA D3 receptors was found in the dorsal striatum in alcohol-preferring HAD and P rats undergoing a similar procedure (Vengeliene *et al*., 2006). This implies that the nigrostriatal DAergic pathway might mediate relapse-like drinking behaviour. Indeed, alcohol and other drugs of abuse produce strong habits that might involve the nigrostriatal pathway known to mediate those behaviours (Dickinson *et al*., 2002; Gerdeman *et al*., 2003; Everitt and Robbins, 2005; Spanagel and Heilig, 2005). Thus, the observed upregulation of striatal D3 receptors after long-term home cage alcohol exposure might not be related to the alcohol intake *per se* but rather to the stimulus–response habit (Vengeliene *et al*., 2006).

Administration of haloperidol, a DA antagonist with selectivity for D2-like receptors, led to a suppression of an ADE in mice (Salimov *et al*., 2000). In addition, a highly selective D3 receptor antagonist caused a dose-dependent reduction of relapse-like drinking in the ADE model in Wistar rats (Vengeliene *et al*., 2006).

5-HT_3 receptor antagonists, known to block alcohol-stimulated DA release in the mesolimbic system, were also able to suppress relapse-like drinking in alcohol-preferring P rats (Rodd-Henricks *et al*., 2000).

Cannabinoids/opioids

González *et al*. (2004) showed that the levels of endocannabinoids underwent significant changes in reward-related areas during relapse with lowest values in this phase. Thus, the induction of compensatory mechanisms, such as an upregulation of the CB1 receptor and a decrease in endocannabinoid levels within the endocannabinoidergic system, might be a determinant for relapse behaviour. In line with this suggestion is the observation that following alcohol consumption with repeated deprivation phases the CB1 receptor displays a long-lasting upregulation (Rimondini *et al*., 2002). In fact the administration of rimonabant—an antagonist acting at the CB1 receptor—has been shown to suppress the ADE in alcohol-preferring sP rats (Gessa *et al*., 2005).

Reduction of an ADE could also be achieved by the use of unselective opioid receptor antagonists (Hölter and Spanagel, 1999; Mormede *et al*., 2004). In contrast, the initial increase in alcohol intake seen after a period of deprivation in high-alcohol-preferring rat lines was enhanced by administration of morphine (Mormede *et al*., 2004). Similar results were obtained with the highly selective κ opioid receptor agonist CI-977 (enadoline). Thus, in long-term alcohol-experienced rats, a strong increase in their alcohol intake during expression of an ADE as a result of CI-977 treatment was seen (Hölter *et al*., 2000b). Several studies in alcohol-dependent patients have linked specific genetic variations within opioidergic genes with alcoholism. Xuei *et al*. (2006), for example, demonstrated an association of the κ opioid system with alcohol dependence.

NPY

Intracerebroventricular infusion of NPY has been shown to reduce alcohol intake after a deprivation phase (Gilpin *et al*., 2003), whereas overexpression of NPY in the amygdala was shown to blunt the increase in alcohol intake following repeated deprivation phases (Thorsell *et al*., 2007). This suggests an important role of this system in relapse to alcohol use.

In summary, the ADE model has been used in the last decade to map the neurochemical substrates underlying relapse-like drinking behaviour. Most evidence comes from pharmacological intervention studies on different glutamate receptors. Findings from these preclinical studies can be directly transferred to the human situation and in fact several new compounds, which have been characterized in the ADE model, are currently undergoing clinical testing for prevention of relapse behaviour (Spanagel and Kiefer, 2008).

Conclusions

Alcohol is known to exert its primary action via a number of CNS neurotransmitter/neuromodulator systems, including the NMDA, $GABA_A$, glycine, 5-HT_3 and nAChRs as well as L-type Ca^{2+} channels and GIRKs. Variations in the genes of these target molecules may result in different alcohol-susceptible phenotypes. In particular, innate differences in the functioning of several brain systems, which define the reinforcing properties of alcohol, contribute to an individual response to this drug. In addition, very recent studies suggest

that appetite-regulating peptides, such as leptin, ghrelin and orexin also play a relevant role in the regulation of the reinforcement system (Jerlhag *et al*., 2006a, 2007; Lawrence *et al*., 2006).

Once alcohol drinking is initiated, alcohol affects virtually all brain neurotransmission. Therefore, it is difficult to define which of these systems contributes most to the transition from controlled to compulsive drug use. However, some of the counteradaptive changes within the brain reinforcement system including the modulatory input systems may become persistent and it is believed that those persistent changes constitute the 'molecular and structural switch' (Spanagel and Heilig, 2005) from controlled alcohol intake to compulsive alcohol abuse. However, those irreversible changes have so far not been clearly identified and it is suggested that in addition to the mesolimbic DA system, other brain systems, including the mesocortical and nigrostriatal pathways as well as their non-DAergic feedback loops and glutamatergic inputs might be involved in alcohol addiction. Furthermore, a persistent recruitment of anti-reward/stress mechanisms such as hypertrophic CRF1 receptor signalling might come into place as well.

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Conflict of interest

RS holds research contracts and is a consultant of several pharmaceutical companies.

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