Turning the Clock Ahead: Potential Preclinical and Clinical Neuropharmacological Targets for Alcohol Dependence

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Abstract: Treating alcohol use disorders represents a main goal in public health, but the effect of current medications is modest. Thus, in the last few years, research has been focusing on identifying new neuropharmacological targets for alcohol dependence. This review will summarize recent research, which has identified new targets to treat alcohol dependence. A variety of systems have been investigated, such as the endocannabinoid system, modulators of glutamatergic transmission, corticotropin-releasing factor (CRF), neuropeptide Y (NPY), nociceptin, glial cell line-derived neurotrophic factor (GDNF), acetaldehyde (ACD), substance P and Neurokinin 1 (NK1) receptor, nicotinic acetylcholine receptors (nAchRs), alpha-adrenergic receptor, and many others. Compared to preclinical studies, only a few clinical studies have been conducted so far. Thus, there is a critical need to translate successful preclinical results into human clinical trials. However, since some clinical studies have failed to replicate preclinical findings, future research will have not only to identify more efficacious medications, but also delineate the best match between a particular pharmacotherapy with a specific alcoholic subtype.

Keywords: Alcohol dependence, neurobiology, pharmacotherapy.

INTRODUCTION

Alcohol induces several neurobiological changes in the central nervous system (CNS). Molecular pharmacology studies demonstrate that alcohol has only a few known primary targets, which include N-methyl-D-aspartic acid (NMDA), γ-aminobutyric acid (GABA), glycine, serotonin, nicotinic acetylcholine (Ach) receptors, calcium (Ca2+) and potassium (K+) channels [1]. The initial exposure of alcohol to specific targets in the brain leads to the typical acute subjective psychotropic effects of this drug, ranging from disinhibition to sedation and even hypnosis, in parallel with increased alcohol concentration. Alcohol also acts indirectly on a variety of neurotransmitter/neuropeptide systems including monoamines such as dopamine (DA), beta-endorphin, and endocannabinoids, and is crucial for the initiation of alcohol reinforcement and reward.

Traditionally, the activity of the mesolimbic DA system plays a crucial role during the initial phase of alcohol consumption responding with an increase in activity after acute alcohol challenge [2]. However, after chronic treatment the DA system appears to reduce its tonic activity [3-5] and these effects are accompanied by similar changes in the human brain [6,7]. Thus, it appears that a “normalization” of DA activity would be desirable, therapeutically speaking. Indeed, the “hypodopaminergic hypothesis” suggests that “boosting” the DA system should ameliorate compulsive drug/alcohol use. Chronic alcohol consumption affects virtually all brain neurotransmitters. These changes likely represent part of the pathophysiological process of alcohol dependence (AD) and contribute to the compulsive use and loss of control over drinking seen in alcoholism. Compulsive alcohol drinking is characterized by a decrease in the function of the reward circuitry and a recruitment of antireward/stress mechanisms, with a hypertrophic corticotropin-releasing factor (CRF) system and a hyperfunctional glutamatergic system [8].

These alcohol-induced neurobiological changes represent possible molecular targets for pharmacotherapies of alcoholism. Pharmacotherapies for alcoholism may either modulate or block the rewarding effects of alcohol or stabilize neurobiological systems dysregulated by chronic alcohol intake, which help to facilitate abstinence or greatly reduce alcohol consumption. Consistent with these concepts, evidence is available that pharmacological treatments can prolong the time to relapse following cessation of heavy drinking, or decrease the number of heavy drinking days [9].

In the United States (US), disulfiram, naltrexone (oral and injectable) and acamprosate are approved medications for the treatment of AD. Several other medications (e.g. topiramate, ondansetron, baclofen, and others) are also under investigation and hold significant promise. While other articles in this special issue review many of these medications [10-16] the aim of this review is to summarize recent research, which attempts to identify new future neuropharmacological targets. These novel targets may eventually lead to the development of new medications for AD.

CANNABINOID RECEPTORS

The endocannabinoid system has an important role in the regulation of the pharmacological and behavioural effects of alcohol, preference, drinking, and vulnerability to relapse [17-19].

The main brain endocannabinoid receptor subtype, the cannabinoid-1 (CB1) receptor, is widely distributed in the CNS, with high density in the brain reward circuitry, including the ventral tegmental area (VTA) and the nucleus accumbens (NAc) [20]. Endocannabinoids that activate CB1 receptors present on axon terminals of GABAergic neurons in the VTA act as retrograde messengers, by inhibiting GABA synaptic transmission, and thus removing this inhibitory input on dopaminergic neurons. Glutamate transmission from neurons of the prefrontal cortex in the VTA and NAc is similarly modulated by the activation of CB1 receptors. Acute alcohol-induced DA release in the NAc is mediated by CB1 receptors and chronic ethanol vapor exposure increases brain endogenous endocannabinoids levels and down-regulate CB1 receptors [20-22]. Thus, the endocannabinoid system is involved in the rewarding effects of alcohol; as alcohol increases dopaminergic neuron firing rates, the release of endocannabinoids in the VTA further enhances dopaminergic activity [23].

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Consistent with the evidence summarized above, enhancement of the CB1 receptor function increases ethanol drinking. For example, acute administration of the CB1 receptor agonists WIN 55,212-2 and CP 55,940, significantly stimulates voluntary ethanol intake in ethanol-prefering sP rats [24]. In contrast, genetic or pharmacological blockade of the CB1 receptor decreases in ethanol consumption. Several studies with CB1 receptor knock-out mice show that genetic CB1 receptor blockade results in a reduction in voluntary ethanol intake [25-29]. Pharmacological blockade with rimonabant, a potent, selective, and orally active blocker of the central CB1 receptors [30] prevents habituation to ethanol drinking, when administered at the start of exposure to alcohol in genetically-selected alcohol-prefering rats [31-34]. Similarly, using different animal models of addiction, the drug rimonabant, in which alcohol intake was already established, rimonabant (administered either acutely or repeatedly) reduced ethanol intake [35-38], and suppressed alcohol self-administration [39-41]. Notably, rimonabant augmented inhibitory post-synaptic potentials, revealing a tonic endocannabinoid activity that decreased inhibitory transmission in central amygdala [42]. Furthermore, experiments employing validated animal models for human relapse (e.g. ‘alcohol deprivation effect’, ‘reinstatement model’) have shown the efficacy of both rimonabant in suppressing relapse in alcohol-prefering animals [43-46], and surinabant, in preventing habituation to ethanol drinking and in showing anti-relapse properties [47-48].

Two human studies testing rimonabant for alcoholism have been conducted. The first study was a Phase 2a, double-blind, placebo-controlled study in which 258 recently detoxified AD patients received a 12-week treatment with rimonabant (20 mg/d) or placebo [49]. This trial did not show any statistically significant difference between the two groups in time to first drink, time to first heavy drink, nor in the cumulative abstinence duration (days of abstinence). The second study was a Phase III, double-blind, placebo-controlled laboratory study aimed at assessing the effect of rimonabant (20 mg/d) on alcohol consumption in 49 non-treatment seeking heavy alcohol drinkers [50]. In the naturalistic (outpatient) phase of the study, rimonabant failed to alter daily alcohol consumption. In the experimental (inpatient) phase of the study, after ingestion of a priming dose of alcohol, participants had the choice to drink up to an additional eight alcohol drinks or receive a small amount of money for each non consumed drink. Again, rimonabant did not significantly alter the number of alcohol drinks consumed during the self-administration experiment.

In conclusion, while animal studies suggest that the CB1 receptor represents a new target for the treatment of AD, human studies do not demonstrate any beneficial effect of rimonabant, compared to placebo, on drinking outcomes. Thus, future studies are needed to investigate the discrepancies between animal and human studies. We also note that there are concerns that the medication may increase depression and suicidality [51].

MODULATORS OF GLUTAMATERGIC TRANSMISSION

Glutamate is the primary excitatory neurotransmitter in the brain and a mediator of the synaptic plasticity required for organisms to adapt behaviour to a changing environment [52,53]. Glutamate, through sensitization and drug-seeking behaviours, plays a crucial role in the pathogenesis of alcohol and drug addiction. In particular, the cycle of alcohol intoxication, withdrawal, abstinence, and relapse induces a hyperglutamatergic state in brain regions associated with alcohol reward, which may contribute in increased alcohol intake and vulnerability to relapse in alcoholics [20].

Glutamate receptors fall into one of two categories: ligand-gated ion channels (i.e., ionotropic glutamate receptors, or iGluRs) and G-protein coupled receptors (i.e., metabotropic glutamate receptors, or mGluRs) [54]. Both groups of glutamate receptors seem to play a role in the neurobiological mechanisms on the basis of alcohol dependence, thus being suggested as potential targets for alcohol pharmacotherapy. Three different types of iGluRs have been identified: the NMDA receptor, the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor, and the kainic acid (KA) receptor. These receptors are ligand-gated ion channels that mediate fast excitatory neurotransmission [54]. Numerous preclinical studies show that GluR ligands alter the reinforcing effects of drugs of abuse, such as ethanol, and can modify drinking behaviour [55]. NMDA antagonists can attenuate the alcohol deprivation effect, cue-induced reinstatement of alcohol-seeking behaviour, acquisition of ethanol conditioned place preference and sensitization to the locomotor stimulant effects of low doses of ethanol [55]. However, Vosler and colleagues [56] showed that NMDA antagonists, such as MK-801, while producing ethanol-like discriminative stimulus effects, did not reinstate ethanol-seeking behaviour. AMPA/KA receptor antagonists also attenuate operant ethanol reinforcement and cue-induced reinstatement in preclinical studies [55]. However, in some of these studies, an effect in reducing sucrose or saccharin reinforcement by NMDA or AMPA/KA ligands has been observed, thus suggesting a non selective action in reducing alcohol intake.

When tested in humans, the majority of iGluR antagonists had significant side effects, including memory loss, disorientation, and psychotic symptoms. Memantine is one of the few NMDA receptor antagonists that is generally well tolerated by humans and does not appear to have abuse potential [57]. Memantine is a more specific non-competitive NMDA receptor antagonist that has been used in Europe for over 20 years to treat neurological diseases. Memantine was approved by the Food and Drug Administration (FDA) in 2003 for the treatment of Alzheimer’s disease. Bisaga and Evans [58] evaluated the acute effects of memantine (15 and 30 mg) in moderate drinkers (20 drinks/week), who had no diagnosis of AD and were not looking for treatment. In this study, memantine (30 mg) reduced alcohol craving before alcohol administration. Krupitsky and colleagues [59] evaluated the effects of memantine (20 mg/d and 40 mg/d) on alcohol craving where alcohol craving was assessed before and after exposure to alcohol cues. Results of this study showed that memantine attenuated alcohol cue-induced craving in a dose-related fashion. Moreover, Krupitsky and colleagues [60] also showed an effect of memantine 10 mg/d in reducing withdrawal symptoms in detoxified alcoholics. However, a double-blind placebo-controlled pilot study with memantine for AD found no differences in drinks per day and drinks per drinking day between the two groups [61]. Indeed, there was a significantly greater percentage of abstinence days in the placebo compared to the memantine group.

The anticonvulsivant levetiracetam blocks ethanol-seeking behaviour by inhibiting AMPA receptors [62]. An open-label study suggested the ability of levetiracetam to decrease alcohol withdrawal symptoms [63]. A second open-label study testing levetiracetam (2000 mg/d) in 20 alcohol-dependent subjects treated for 10 weeks, reported a reduction in alcohol drinking, craving and addiction severity [64]. While double-blind placebo-controlled studies are needed to draw any conclusion, the preliminary evidence highlights the safety of levetiracetam, when administered to AD individuals.

Given the side effects observed with the majority of iGluRs ligands, there is interest in selective mGluRs ligands, which mediate slower, modulatory glutamate neurotransmission [54]. Attenuation of glutamatergic transmission by mGluRs ligands reduces the rewarding and reinforcing effects of ethanol as well as relapse-like behaviours [55]. There are eight different mGluRs, which belong to three groups, based on sequence similarities, preferred signal transduction pathways and pharmacology. Specifically, these groups are Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) [65]. mGluR5 and mGluR2/3 receptors are widely distributed in meso-
corticolicorticobrillar brain regions associated with drug reinforcement. In particular, there is a high expression of mGluR5 in forebrain and limbic structures (olfactory bulb, anterior olfactory nucleus, olfactory tubercle, dorsal striatum, NAc, lateral septum, hippocampal formation). mGluR5s are located at both presynaptic and postsynaptic sites in the brain and it has been observed that the activation of mGluR5s interacts with ionotropic NMDA receptors, enhancing NMDA responses in striatal projection neurons [66]. This last observation suggests that blockade of mGluR5s could reduce glutamatergic transmission through NMDA receptors. Thus, blockade of mGluR5s could attenuate the state of hyperexcitability induced by glutamatergic excitatory neurotransmission during alcohol withdrawal and abstinence, which may be associated with a high risk of relapse [20,67].

In 1999, Gasparini and colleagues [68] published the structure and initial characterization of a highly potent, non-competitive mGlu5 receptor antagonist, the 2-methyl-6-(phenylethynyl)pyridine (MPEP). Later studies showed that MPEP also acts as an antagonist of the NMDA receptor and inhibits monoamine oxidase-A [69]. The effects of MPEP on voluntary ethanol consumption and relapse behaviour were tested in preclinical studies. MPEP significantly attenuates ethanol seeking in alcohol-prefering rats in a dose-related manner. Moreover, treatment with MPEP (twice a day) resulted in a significant dose-dependent reduction of the alcohol deprivation effect and to a lesser extent, of baseline drinking [70].

A possible explanation for the ability of mGlu5 negative allosteric modulators (NAMs) to reduce ethanol self-administration is that mGluR5 NAMs alter the discriminative stimulus effects of ethanol [71]. The lack of selectivity of MPEP combined with undesirable pharmacological properties (e.g. low water solubility and low brain penetration) led Cosford et al. [72] to develop a new mGlu5R antagonist, the 3-[2-methyl-1,3-thiazol-4-yl]ethynylpyridine (MTEP), which holds a greater selectivity for the mGlu5 receptor and greater bioavailability. MTEP was tested in different rodent models of alcoholism and it reduced alcohol-seeking behaviour, decreasing both the consumption of ethanol and the appetitive response for ethanol [73,74]. The effects of MTEP are associated with regulation of cortical glutamate systems, particularly those in olfactory-related regions [73,74].

Besheer and colleagues [75] suggest that mGluR1 antagonists, unlike mGlu5R antagonists, do not produce alcohol-like interoceptive effects and do not alter the interoceptive effects produced by alcohol. The two receptors mGlu1 and mGlu5 are both located primarily at postsynaptic sites, and share G-protein and cell signalling mechanisms. A functional dissociation between mGlu1 and mGlu5 receptors in the interoceptive effects of alcohol may represent an explanation of the different effects of the antagonism at mGlu1 vs. mGlu5 receptors [75].

Members of the Group II mGlu receptors (mGlu2/3) have also emerged as potential therapeutic targets for treating alcohol AD. Group II agonists have anxiolytic and anticonvulsant properties and preclinical studies demonstrate that mGluR2/3 agonists reduce active drug reinforcement and drug-seeking behaviour [54]. mGlu2/3 receptors are expressed preferentially in mesocorticolicorticobrillar brain regions and are located both pre- and post-synaptically. Presynaptic autoreceptor function is implicated in its therapeutic action as mGlu2/3 receptor agonist reduces extracellular glutamate levels in the NAc, whereas receptor blockade increases extracellular glutamate [20]. Backström and Hyttä [76] tested the effects of a pretreatment with the mGlu2/3 receptor agonist LY379268 in attenuating alcohol-seeking behaviour and alcohol self-administration in rats. This experiment showed that LY379268, used at different doses [0, 1, 3 and 5 mg/kg intraperitoneally (i.p.)], attenuated alcohol self-administration and reinstatement at doses that also decreased spontaneous locomotor activity. Of note, similar effects were found for (S)-3,4-DCPG, an antagonist of the mGlu8 receptor (Group III) [76]. Subsequently, Rodd and colleagues [77] demonstrated that LY404039, suppressed reinstatement of ethanol-seeking behaviour induced by cues but not active ethanol self-administration. It has been suggested that the reason for the lack of effect of LY404039 on ethanol self-administration may be the use of a high ethanol-prefering P rat strain as opposed to outbred rats used in other studies [54]. mGluR2/3 agonists, in particular LY379268, modify ethanol-seeking induced both by stress and exposure to drug-related environmental stimuli, suggesting that mGlu2/3 receptors participate in mediating the effects of contextual stimuli conditioned to drugs [78].

However, some of the above-mentioned effects of mGluR2/3 agonists on drug and ethanol reinforcement or reinstatement must be interpreted with caution, as the effects of mGluR2/3 agonists on ethanol consumption may not be direct, but secondary to other effects [54].

These data from animal studies suggest that Groups I and II mGluRs may represent promising treatment targets for stress- and cue-induced alcohol seeking and relapse. However, there are no human studies on the efficacy of either Group I mGluR NAMs or Group II mGluR agonists in the treatment of AD.

Corticotropin-releasing factor (CRF) and CRF-like peptides

Stress activates a number of biological systems in order to appropriate a coordinated physiological and behavioural response. Two important biological stress systems, the hypothalamic-pituitary axis (HPA) and the locus coeruleus/norepinephrine system (LC-NE) interact to exert their coordination of the appropriate behavioural response. Individuals cope with stress differently. Aberrant coping mechanisms in response to stress have been linked to a wide range of psychiatric disorders such as depression, anxiety and drug and alcohol dependence [79,80]. In particular, the stress response has not only a key role in the development and facilitation of alcohol and substance abuse disorders [81], but it is also implicated in a behavioural component of alcohol relapse [82].

Corticotropin-releasing factor (CRF) also called corticotropin-releasing hormone (CRH), represents one of the most intensively studied stress-related peptides in the field of addictions. CRF is a 41 amino acid peptide, secreted from the paraventricular nucleus (PVN) of the hypothalamus in response to stress. CRF is expressed in neuroanatomical regions associated with substance abuse and reward such as the central nucleus of amygdala (CeA) [83], the LC and the hypothalamus. Moreover, CRF appears to link two of the main stress response systems, namely the HPA-axis and the LC-NE system. This suggests a central role for CRF in coordinating and controlling many of the different afferents of the biological stress pathways and their role in substance abuse [82-84]. Preclinical studies demonstrate that acute ethanol withdrawal increases CRF levels in the CeA [85] and in the bed nucleus of the stria terminals [86] mediating stress and anxiety responses. These responses are reversed by non-selective CRF antagonist administration [87]. CRF exerts its effects on cells by binding the CRF1 and CRF2 receptors [88], which are members of the 7 transmembrane G-protein coupled receptor family. CRFR1s are found at high levels in the cerebral cortex, cerebellum, amygdala, hippocampus and olfactory bulb [89] and mediate anxiogenic properties of CRF and the coordination of the stress response [90]. In fact, CRFR1 null mice show decreased anxiety like behaviour and display an impaired stress response [91]. The role of the CRFR2 still remains controversial, as there is evidence from animal models suggesting CRFR2 antagonists have an anxiolytic effect [92], however CRFR2 null mice display an anxiogenic phenotype [93].

In the genetically selected msP rats, high alcohol preference has cosegregated (the mutated gene and the disease are transmitted together to the next generation) with increased behavioural sensitivity to stress, creating a phenocopy of the postdependent phenotype. For example, Hansson et al. [94] identified a genetic variant in the
promoter region of CRFR1, which resulted in higher CRFR1 expression in several brain regions of the msP line and was associated with increased sensitivity to relapse into alcohol seeking induced by environmental stress [94]. In the same animal model, Hansson et al. [94] measured CRFR1 transcript in CeA, medial nucleus of amygdala (MeA) and NaAc after two weeks of exposure to alcohol. In these areas, a down regulation of CRFR1 transcript was found, suggesting that alcohol could be voluntarily consumed by these animals partially for the ability of alcohol to reduce CRFR1 activity [95].

Similarly, an upregulation of the CRFR1 transcript was present within the basolateral and medial amygdala in genetically non-selected rats following a history of dependence. This upregulation probably contributed to their behavioural phenotype with elevated voluntary alcohol consumption and potentiated stress sensitivity during protracted abstinence. Moreover CRFR1 antagonism eliminated this increased behavioural sensitivity to stress in the postdependent state [96].

In addition to the large body of evidence that upregulated activity of the CRF system confers susceptibility for excessive self-administration of alcohol and relapse, there are also data linking genetic polymorphisms of CRF receptors to drinking behaviour in humans. For example, two tagging SNPs (htSNPs) of the CRF1 gene, rs242938 and rs1876831, were analyzed in a cohort of adolescents with little previous exposure to alcohol and a cohort of AD adults [97]. This study linked genetic polymorphisms in CRF1 to risky-drinking behaviour in adolescents. In fact, there was an association of SNPs rs242938 and rs1876831 with binge-drinking, lifetime prevalence of alcohol intake and lifetime prevalence of drunkenness. In addition, the authors found an association between rs1876831 and high amount of drinking in AD adults. Blomeyer et al. [98] measured the effects of negative life events on the amount of drinking in the same cohort of adolescents genotyped for CRF1 htSNPs by Treutlein et al. [97]. In this study, the C allele of htSNP rs1876831 was associated with drinking more alcohol per occasion and increased lifetime rates of heavy drinking, and this was mediated by the number of negative life events in the last 3 years [98].

While more research is needed to confirm this finding, the impact of stress on heavy drinking could be more evident among individuals carrying a particular genotypic variant of CRF1 gene. It is possible that these CRF1 polymorphisms [97] may be predictive of response to CRF1 antagonist treatment for alcoholism, an intriguing pharmacological approach to treat AD. The CRF1 receptor seems to mediate the enhancement in ethanol self-administration during abstinence period in AD models like C57BL/6J mice. In fact, the increase in ethanol self-administration is less in CRFR1 knockout mice after dependence induction. Moreover, the CRF1 antagonist antalarmin reverses the enhanced self-administration in this animal model [99]. CRF1 antagonists selectively reduce excessive self-administration of ethanol in alcohol dependent rats also during acute withdrawal, while the same antagonism has no effect on ethanol self-administration in nondependent animals [100].

Another CRF1-receptor antagonist, (N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-7-amine), MPZP, blocked dependence-induced increases in alcohol drinking by P rats without affecting operant alcohol responding in nondependent controls [101].

The newly synthesized CRF1R antagonist 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethylimidazo[1,2-b]pyridazine (MTIP) is a novel, orally available and brain-penetrant CRF1 antagonist, which showed in vivo activity in preclinical models of alcoholism. Compared to other well-characterized CRF1R antagonists, MTIP had a markedly reduced volume of distribution and clearance. MTIP blocked excessive alcohol self-administration in Wistar rats with a history of dependence, as well as in a genetic model of high alcohol preference, the msP rat, at doses that had no effect in nondependent Wistar rats. Also, MTIP blocked reinstatement of stress-induced alcohol seeking both in postdependent, and in genetically selected msP animals, again at doses that were ineffective in nondependent Wistar rats [102,103].

Recent studies indicate that CRF is not the sole ligand of CRF receptors, and that the endogenous neuropeptides urocortin 1 (Ucn1), urocortin 2 (Ucn2, also known as stresscopin related peptide) and urocortin 3 (Ucn3, also known as stresscopin) also bind these receptors [104-106]. Moreover, these studies show that all three urocortins have a higher affinity than CRF at CRF2. CRFR2 is currently under investigation in AD. The lateral septum (LS) and dorsal raphe (DR) are major loci of CRFR2s [106].

Ucn1 binds both CRF receptors with the highest affinity. Ucn1 in the brain is primarily expressed in the pericurolomotor urocortin-containing population of neurons (pIIu), a brain region thought to be only involved in ocular function [107]. The projections from the non-preganglionic Edinger-Westphal (npEW) nucleus include a large number of brain regions. The most prominent projections include the LS, the DR and spinal cord [108,109].

Although the regulation of Ucn1 expression is not completely studied or understood, results from the majority of inducible transcription factors (ITFs) mapping studies indicate that the npEW is one of the brain areas most sensitive to ethanol, and that it is persistently activated following repeated alcohol self-administration [110-112]. In summary, in animal models the Ucn1/CRF2 receptor system expression seems to be modified by prolonged ethanol exposure suggesting that this system may be involved in behavioural changes related to AD [112].

The Ucn1 system is not only sensitive, but also may be involved in the genetic predisposition to high alcohol intake, as a number of studies evaluating the Ucn1 strains levels show higher Ucn 1 measures in rodents lines selectively-bred to prefer alcohol versus alcohol avoiding rats [123-128].

Furthermore, manipulations of the Ucn1 neurocircuit modulate alcohol consumption and sensitivity. Mice with successful npEW lesions were compared to sham-operated controls and dramatic effects of these lesions on alcohol consumption were observed. Mice with npEW lesions significantly reduced their preference for alcohol when exposed to a choice between water and 3%, 6% or 10% ethanol [129].

The Ucn1/CRF2 neurocircuits have no apparent connections to the mesolimbic DA system and the amygdala. Moreover, while the DA system is thought to play an important role in the appetitive phase of reinforcement [130], there is no evidence for the involvement of the Ucn1 system. Therefore, it is more likely that the npEW is involved in regulation of ethanol intake through effects on the consummatory phase of reinforcement. This regulation is most likely to occur through from the npEW to other brain regions. LS could be a target of npEW projections, which are involved in the regulation of this behaviour. Recent studies show that Ucn1 acts to depress glutamatergic transmission in the LS through CRFR2 [131-132]. These studies lead to hypothesize a simplified neurocircuit in which activation of the npEW by ethanol leads to inhibition of activity of the LS (perhaps through inhibiting the glutamatergic excitation of this brain area). Since LS neurons are primarily GABAergic, their inhibition by Ucn would lead to disinhibition of hypothalamic target brain regions, and thereby increased to consumption of available fluids like ethanol. Clearly, additional studies are needed to fully address this hypothesis. One of the missing links in this scheme is the location of hypothalamic brain regions disinhibited through LS, and contributing to fluid intake. The hypothalamus is a long-proposed integrator of consummatory responses, but hypothalamic subregions affected by ethanol have not been mapped in sufficient detail [133]. A recent study by Ryabinin et al. [134] confirmed the importance of Ucn1 innervation and in particular of the
LS in the regulation of alcohol consumption. In a series of experiments the authors injected Ucn1 or CRF bilaterally at various doses into the lateral septum of male C57BL/6J mice and immediately after the injection tested ethanol consumption. The authors used a limited access procedure, which takes advantage of higher levels of activity, fluid and alcohol consumption that are observed in mice during early hours into the dark portion of the circadian cycle. Injection of Ucn1 into the LS of C57 mice significantly attenuated ethanol consumption both in the expression phase of ethanol drinking and during the acquisition phase of alcohol consumption [134].

One potential explanation for this apparent discrepancy is that if Ucn1 contributes to a reward signal associated with ethanol, exogenous application of Ucn1 could signal that the reward already has been achieved, resulting in no further effort to obtain it (by alcohol consumption). As an alternative explanation, since pIIla neurons contain other peptides besides Ucn1 as potential regulators of the rewarding properties of drugs of abuse, it is possible that synergistic actions of these peptides are needed to increase the rewarding properties of alcohol [134]. It is important to note that Ucn3 innervates the same brain structures and alcohol consumption may also be regulated by this peptide. In particular the treatment of AD rats with urocortin III reduced anxiety and ethanol self-administration in the early stages of withdrawal [135].

Although this emerging evidence strongly suggests that CRF receptors represent key pharmacological targets for AD, a clinical concern and potential limitation is that CRF1 and CRF2 agents might have undesirable hormonal and affective side effects, like impaired stress responses and abnormal anxiety-like behaviours [136-138].

**NEUROPEPTIDE Y (NPY)**

Neuropeptide Y (NPY) is a 36-amino acid peptide that is expressed at high levels within the mammalian nervous system. NPY is one of the most abundant neuron modulators in the CNS and is considered an important regulating factor in emotional behaviour. For example, administration of exogenous NPY has anti-anxiety and sedative effects that rely, at least partially, on activation of Y1 receptors in the amygdala [139-141]. The effects of NPY are mediated at Y1, Y2, and Y5 receptors, of which there are currently four subtypes identified: Y1, Y2, Y4, and Y5 with Y1 and Y2 receptor subtypes found at significant levels in the CNS. Antidepressant-like actions of NPY have been reported in rats [142], and Y1 receptors seem to mediate this effect [143-144]. NPY-Y2 receptors also play a role in the regulation of emotion, as they are located presynaptically on NPY-ergic neurons and control the release of endogenous NPY [145].

In animal models, acute and repeated stress exposure induces different effects in the central expression of NPY. An acute stressor significantly decreases NPY expression in the amygdala, an effect that is concurrent with the anxiogenic effects of stress. On the contrary, when the stressor is repeated, NPY expression is increased in the amygdala, correlated to a behavioural adaptation of stress [146-147]. As a consequence, NPY expression seems to be involved in the behavioural adaptation to stressors [146-147].

Using genetically modified mice, NPY levels were inversely related to ethanol intake [148]. As NPY levels are lower in the CeA and MeA of alcohol preferring (P) rats compared to non P (NP) rats, NPY infusion normalizes the decreased expression of NPY in the CeA phosphorylation and attenuates the anxiety-like and alcohol drinking behaviours of P rats. Thus, a deficiency in NPY signaling in the CeA may be involved in regulating both anxiety and alcohol drinking behaviours [149] and that NPY system modifications can influence ethanol intake [150-153]. Notably, intramygdala administration of a viral vector designed to over-express NPY was found to have anxiolytic-like properties and to suppress ethanol intake in rats [154]. Moreover, intracerebroventricular (i.c.v.) infusion of NPY in rats has the ability to prevent stress-induced relapse, by blocking, dose-dependently, the reinstatement of alcohol seeking induced by a pharmacological stress (yohimbine i.p.) [155]. Together, these preclinical studies suggest that in particular the NPY-Y1 receptor, may represent a novel pharmacological target for alcoholism. Research has also focused on the possibility of the Y2 receptor to affect alcohol drinking. For example, the Y2 antagonist BII0246 suppresses alcohol intake in both naive [156] and postdependent animals [157].

In summary, targeting the NPY system, possibly through antagonism at presynaptic Y2 autoreceptors, may offer an attractive strategy for developing novel antidepressant and anti-anxiety treatments that may also affect ethanol intake.

**NOCICEPTIN**

Nociceptin, also called Orphanin FQ (N/OFQ), is a 17-amino-acid peptide that shows structural homology with opioid peptides, particularly dynorphin A [158]. N/OFQ has a high affinity with the opioid receptor-like 1 (ORL-1), now included in the opioid receptor family and renamed the NOP receptor (nociceptin/orphanin peptide receptor). N/OFQ and its receptor are widely distributed in the CNS, including areas involved with the neurobiology of addictions. N/OFQ has been found to act in the brain as a functional antiopioid peptide by blocking opioid-induced supraspinal analgesia [159-160], morphine-induced conditioned place preference [161], and morphine induced increases in extracellular DA levels in the NAc [162]. Moreover, N/OFQ inhibits stress-induced ethanol seeking and exerts general anti-stress-like effects by acting as a functional antagonist of extrahypothalamic CRF transmission [163].

Genetic variants of the N/OFQ and NOP genes may be linked to vulnerability for alcoholism. One study revealed a link between alcoholism and two SNPs in the N/OFQ gene [164]. Moreover, the SNP rs6010718 of the gene encoding the NOP receptor was associated with the diagnosis of AD in a Scandinavian sample [165].

Several studies demonstrate that N/OFQ regulates ethanol preference, ethanol reward, and ethanol-seeking behaviour. For example, activation of NOP receptors by N/OFQ inhibits home cage ethanol drinking and operant ethanol self-administration. N/OFQ also reduces both ethanol-induced conditioned place preference and conditioned reinstatement of alcohol seeking [166-168]. Moreover, N/OFQ inhibits stress-induced ethanol seeking and exerts general antistress effects by acting as a functional antagonist of extrahypothalamic CRF transmission [169-170]. Studies using alcohol preferring msp rats demonstrated that subchronic administration of N/OFQ or NOP analogs significantly reduce ethanol self-administration [171-172]. By contrast, in non selected Wistar rats tested under the same experimental conditions, N/OFQ did not alter ethanol consumption [173]. Studies with in situ hybridization revealed higher expression levels of N/OFQ and NOP receptor mRNA in numerous brain areas of msP compared to Wistar rats [173].

Recent data show that the opioid agonist/partial agonist buprenorphine reduces alcohol intake in msP rats via activation of NOP receptors [174]. This last observation not only strengthens the link between N/OFQ and excessive alcohol consumption, but also suggests potential clinical applications. This is consistent with the observation that buprenorphine reduces not only opiate use but also alcohol intake in heroin dependent subjects [175]. In summary, these data provide the rationale for the development of N/OFQ receptor agonists as new pharmacotherapies for the treatment of AD.

**SUBSTANCE P AND NEUROKININ 1 (NK1) RECEPTOR**

Substance P (SP) is an 11 amino acid peptide [176] involved in C-fiber sensory transmission wherein blockade of the transmission of SP produces analgesic and anti-inflammatory effects [177]. Substance P and its receptor Neurokinin 1 receptor (NK1R) are highly expressed in brain regions involved in drug reward and affective
behaviours (for review see [178]). NK1R agonists have anxiogenic-
like effects and cause conditioned place aversion. Conversely, 
NK1R agonists produce anxiolytic-like and antidepressant-like 
effects [178] as human studies demonstrate that antagonism of SP 
or of NK1R to be effective for depression or anxiety [179-181]. 
Despite this early evidence of efficacy in treating affective disor-
ders by NK1R antagonists, no further studies were published.

More recently, there has been an interest in the role of NK1R as 
a potential pharmacological target for alcoholism. This interest was 
based on the preclinical evidence that genetic deletion of NK1R 
suppresses alcohol intake [182]. Conversely, inactivation of NK1R 
but not NK1R antagonism critically seems to modulate alcohol 
reward and escalation of alcohol intake following multiple depriva-
tions, two important characteristics of addiction.

In humans, a recent study tested the high-affinity selective 
NK1R antagonist LY686017, a compound characterized by oral 
availability and brain penetration [183]. LY686017 was adminis-
tered daily to 50 recently detoxified patients with a diagnosis of AD 
and with high trait anxiety [Spielberg Trait Anxiety Inventory Test 
(STAI) > 39]. LY686017 was administered at the dose of 50 mg 
daily, which yields a > 90% blockade of central NK1R. LY686017 
was significantly more effective than placebo in suppressing sponta-
eous alcohol craving, improving overall well-being, blunting 
cravings induced by a laboratory challenge procedure, and attenuat-
ing cortisol response. Finally, an examination of allelic variations in 
the NK1R gene in 271 alcoholic subjects and in 337 healthy con-
trols found that the development of AD in Caucasian individuals 
is significantly associated with some polymorphisms of the NK1R 
gene [184].

In summary, preclinical and clinical evidence suggest that 
NK1R antagonism might be of therapeutic value in alcoholism. 
Future studies will have to identify the best population responding 
to NK1 R antagonism. In this regard, considering that Substance P 
acts at NK1Rs to mediate both behavioural stress responses and 
drug reward mechanisms, the NK1R antagonist aprepitant is cur-
tently under investigation for the treatment of alcoholics with Post-
traumatic Stress Disorder (PTSD) co-morbidity (NCT00896038).

NEUROKININ B AND TACHYKININ RECEPTORS

Similar to NK1 receptors, tachykinin receptors 3 (NK3Rs), the 
receptors for the tachykinin 3 peptide (neurokinin B) are involved in 
drug reward mechanisms. In particular, it was hypothesized that 
NK3R agonists reduce ethanol consumption by replacing the re-
warding properties of alcohol, thus making alcohol consumption 
redundant [185, 186]. A preclinical study in Sardinian preferring 
and non-preferring rats reported that administration of the selective 
NK3R agonist sentkide inhibits alcohol intake, without affecting 
overall water or food intake [187, 188]. The brain regions that were 
highly sensitive to the inhibitory effect of the tachykinin NK3R 
agonists were the lateral hypothalamus and the nucleus basalis 
magnocellularis [189].

Because of mouse, rat and human species differences in the 
NK3R, no clinical trials have been carried out to evaluate the effi-
cacy of NK3R agonists in reducing ethanol consumption in humans 
[190]. However, a recent study within the Collaborative Study on 
the Genetics of Alcoholism (COGA) found evidence of an associa-
tion between a variation in TACR3 gene and susceptibility for AD 
[190]. TACR3 belongs to a family of genes that encode proteins 
that function as receptors for tachykins (including NK3R for neu-
kokinin B). Interestingly, a secondary analysis showed that variation 
in TACR3 was associated with more severe alcoholism [190].

Unlike NK1 and NK3 receptors, tachykinin NK2 receptors are 
not highly abundant in the CNS, although NK2 receptor mRNA and 
NK2 receptor binding sites have been identified in the human brain 
[191]. The selective NK2R antagonist saredutant (SR48968) s has 
anxiolytic- and antidepressant-like activities [192]. However, 
NK2Rs seem to be less involved in reward processes. For example, 
a study by Cicciocippolo and colleagues [193] showed that the NK2 
selective agonist GR 64349, unlike the NK3 selective agonists, did 
not inhibit alcohol intake in Sardinian alcohol-prefering rats. How-
ever, no further studies have been conducted to confirm these find-
ings.

NEUROPEPTIDE S

The 20 amino-acid peptide Neuropeptide S (NPS) and its recep-
tor NPSR were first identified in 2004, as a novel transmitter sys-
tem, mainly expressed in the brain [194]. NPS is co-expressed with 
extSirmitary transmitters such as glutamate, acetylcholine or CRF. 
While the projection areas of these NPS-producing neurons are 
currently unknown, however, outside of the CNS, the NPS precur-
sor is expressed mainly in endocrine tissues [195]. The NPSR is 
a typical member of the G-protein-coupled receptor superfamily and 
is expressed in brain areas involved in olfactory processing, in brain 
areas critical for fear processing (e.g. amygdala and paraventricular 
nuclei of the hypothalamus), as well as in brain regions involved in 
sleep-wake modulation (e.g. thalamic intralaminar nuclei, preoptic 
nucleus or tuberomammillary nucleus). Thus, the NPS system me-
diates specific effects on synaptic transmission to and within the 
amygdala, which are important for processing of acute fear as well 
as extinction of fear memories [196]. Ruggeri et al. [197] investi-
gated the variations of NPSR mRNA expression in rats exposed to 
6 days of intermittent ethanol intoxication and demonstrated an 
increased NPSR mRNA expression in different brain areas of post-
dependent rats. Central activation of NPS receptors facilitates re-
slave to alcohol seeking. Thus, considering that postdependent rats 
are more inclined to relapse, one could speculate that this over ex-
pression may also be functionally relevant in alcohol drinking 
[198]. Another recent experiment sought to determine whether the 
NPS system might play a role in the regulation of alcohol-related 
behaviours [199]. This study showed that i.c.v. NPS does not mod-
ify ongoing ethanol self-administration in rats, indicating that moti-
vational circuitry of ethanol reinforcement is not directly affected 
by activation of NPSR. On the other hand, recent data by Badia-
Elder et al. [200] showed that i.c.v. NPS injection signifi-cantly 
reduces ethanol drinking in genetically selected alcohol preferring P 
rats. Experimental design differences may explain the discrepant 
results of the two studies. Badia-Elder et al. [200] tested the effect 
of NPS on home cage voluntary ethanol drinking, whereas Cannella 
et al. [199] studied peptide effects under operant conditions. Oper-
ant self-administration is thought to more directly gauge motivation 
to obtain the reinforcer, whereas home cage drinking can also be 
controlled by taste, calories and other factors not directly related to 
pharmacological reinforcement. Furthermore, the P rats show elev-
ated anxiogenic-like behaviour and it has been hypothesized that 
their excessive ethanol drinking is in part driven by reduction of 
anxiety symptoms. Notably, NPS has anxiolytic-like effects in ani-
mal models of anxiety [201, 202].

Moreover, Cannella and colleagues [199] showed a three-fold 
increase of relapse in rats subjected to repeated reinstatement test-
ing. As the lateral hypothalamus (LaH) is traditionally implicated in 
reward and motivation and is one of the brain areas with the highest 
NPSR transcript expression levels, Cannella et al. [199] demon-
strated that intra-LaH administration dramatically reinstated extin-
guished ethanol responding suggesting that the LaH is an important 
brain site of action for NPS effects on alcohol relapse.

GALANIN

Galanin is a feeding-stimulatory peptide with neuroprotective 
effects. In fact, increased galanin concentrations seem to have neu-
roprotective effects and promote neurogenesis [203]. The three 
galanin receptors (GαR1, GαR2, GαR3), are involved, centrally, 
in the control of feeding, alcohol intake, seizure threshold, cogni-
tive performance and mood, and peripherally, in the control of pain
threshold [203]. Both human genetic and behavioural animal studies have suggested that galanin may be involved in addictive behaviours, such as repeated alcohol intake. Earlier studies showed that hypothalamic injection of galanin increases the release of DA in the NAc, just as systemic alcohol does [203]. In humans, a genetic study found a significant association between the single nucleotide polymorphism (SNP) rs3091367 of the GaI3R and alcoholism, suggesting that galanin's influence on alcoholism vulnerability may be mediated mainly through GaI3R [204]. This finding suggests that the development of galanin receptor ligands, in particular GaI3R antagonists, might be useful in the treatment of AD.

**GHRELIN**

Ghrelin is a 28-amino acid gut peptide with an n-octanoylation modification at Ser 3 [205]. Ghrelin acts as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R), a G-protein coupled receptor that induces growth hormone (GH) release from the pituitary [205]. Ghrelin’s discovery was related to its ability to stimulate GH release. Subsequently, it has clearly demonstrated that ghrelin affects food-seeking behaviour, stimulating appetite and food intake. Ghrelin stimulates appetite by acting on the hypothalamic arcuate nucleus (ARC), a region that controls the intake of food and other substances, including alcohol. Opioidergic neurons, which play a role in the reinforcing effects of alcohol, also are located in the ARC [206]. In addition to the ARC, GHS-Rs are also highly expressed in the caudal brain stem, the VTA, hippocampus, substantia nigra, and dorsal and medial raphe nuclei [207, 208]. The expression of the GHS-R in the mesolimbic DA pathway suggests that ghrelin could also modulate the hedonic properties of food and the so-called reward system [209, 210]. This hypothesis has been tested in animals. Intracerebral ventricular (i.c.v.) ghrelin administration into the third ventricle increases extracellular concentrations of accumbal DA in NMRI mice [209], also showing that ghrelin activates the cholinergic-dopaminergic reward link and that ventral tegmental nAChRs have a central role for the DA-enhancing properties of ghrelin. A second set of experiments extended the previous results, injecting ghrelin directly into the VTA [211]. These findings were replicated when ghrelin was injected peripherally [212]. A more recent set of experiments used an elegant approach of genetic (GHS-R1A knockout mice) and pharmacological (two GHS-R1A antagonists: BIM28163, delivered i.c.v.; and JMV2959, i.p.) models of suppressed ghrelin signaling [213]. These experiments demonstrated that: i) alcohol-induced brain reward parameters, such as enhanced extracellular accumbal DA overflow, locomotor stimulation and conditioned place preference (CPP) were consistently abolished or attenuated by two GHS-R1A antagonists in wild-type mice and were abolished in GHS-R1A knockout mice; ii) i.c.v. administration of ghrelin to mice significantly increased alcohol consumption compared to vehicle treatment in a 2-bottle (alcohol/water) free choice limited access paradigm. Bilateral administration of ghrelin into either the VTA or the LDTg also increased alcohol consumption in comparison to vehicle. The percentage increase in alcohol consumption was significantly greater following administration to the VTA or the laterodorsal tegmental nucleus (LDTg) compared to the i.c.v. route. Food intake (normal chow) was increased by i.c.v. ghrelin administration in comparison to vehicle but was not affected by bilateral ghrelin administration into either the VTA or the LDTg. Alcohol intake in the 2-bottle (alcohol/water) free choice limited access paradigm was suppressed in mice by both the GHS-R1A antagonists (delivered either i.c.v. or i.p.). The effects of i.c.v. ghrelin on alcohol intake were absent in GHS-R1A knockout mice.

Human studies also shed light on the acute effects of alcohol on ghrelin as well as on the possible role of ghrelin in alcohol-seeking behaviour. In alcoholics, alcohol is able to acutely inhibit ghrelin secretion and plasma ghrelin levels correlate with psychometric determinations of the alcohol seeking behaviour, such as alcohol craving (for review, see [214]). Together, these findings suggest that ghrelin system plays a role in AD, thus representing a new possible pharmacological target.

**GLIAL CELL LINE-DERIVED NEUROTROPIC FACTOR (GDNF)**

The glial cell line-derived neurotrophic factor (GDNF) is a distant member of the transforming growth factor β superfamily that was originally isolated from the rat B49 glial cell line. High levels of the growth factor are found in the striatum (dorsal striatum and NAc), thalamus, cortex and hippocampus. The major source of GDNF in the midbrain is the striatum where GDNF is retrogradely transported by dopaminergic neurons of the substantia nigra pars compacta (SNc) and the VTA [215]. GDNF plays an essential role in the development and survival of motor neurons, in the synaptogenesis and is an essential factor for the maintenance and survival of adult DA neurons. Moreover GDNF seems to regulate neuronal excitability and transmitter release [216].

Dopaminergic neurons within the VTA are a critical component of the neural circuitry implicated in addictive behaviours [217]. GFRα1 and transferrin receptor (FRα) are highly expressed in VTA dopaminergic neurons and increasing evidences suggest that GDNF plays a regulatory role in the actions of drugs of abuse, including ethanol.

Recent studies showed that GDNF inhibits ethanol-drinking behaviours. A single administration of GDNF into the VTA of rats results in a rapid reduction of operant self-administration of ethanol [218, 219]. Moreover the administration of GDNF into the VTA reduces consumption of moderate levels of ethanol in a paradigm that resembles social drinking [218, 219]. On the other hand, in models of excessive and “binge-like” drinking of ethanol (i.e. using a 20% ethanol solution), GDNF infusion in the VTA also reduces ethanol consumption by lever presses [219, 220].

Considering an animal model of relapse, Carnicella and colleagues [219] found that activation of GDNF pathway in the VTA reduces self-administration of ethanol. Together, these results suggest that GDNF is a potent, rapid inhibitor of excessive consumption and relapse to ethanol.

The main signaling pathways activated via GDNF-mediated Ret activation are the MAPK, PI3K and PLCγ. In the VTA, a specific inhibition of MAPK blocked GDNF-mediated reduction in ethanol self-administration [219]. Thus, the MAPK seems the specific signalling pathway by which GDNF mediates a reduction in ethanol-drinking behaviours. GDNF rapidly inhibits the activity of A-type K+ channel currents in primary midbrain neurons via a mechanism that requires the activation of the MAPK pathway [221]. Therefore, it is possible that GDNF, via the inhibition of an A-type K+ channel, alters the excitability of the neurons in the VTA, leading to the decrease in ethanol self-administration.

On the other hand, the long-lasting actions of GDNF may be explained by GDNF-induced transcriptional changes, that could persist beyond the activation and termination of GDNF signaling. [222]. Moreover GDNF upregulates its own expression, leading to a prolonged activation of the GDNF signaling pathway [223]. In addition, several studies suggest a role for GDNF in the regulation of tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of DA in the midbrain [220]. Upregulation of TH levels has been reported as one of the hallmarks of biochemical adaptations to in vivo chronic exposure to drugs of abuse, including ethanol [224].

All these data suggest that GDNF plays an important role in controlling ethanol drinking behaviours and suggest that upregulation of the GDNF pathway may be an approach to treat AD. However, it is unlikely that GDNF itself could be used as a therapeutic agent considering that it cannot cross the blood-brain barrier [225]. Some recent studies suggest the possible use of small molecules that increase the production of GDNF or activate its receptors.
There are several small molecules that have been reported to increase the expression of GDNF or activate the GDNF signalling pathway. Ibogaine is a natural alkaloid reported to reverse the adverse actions of multiple drugs of abuse including alcohol [226]. Systemic administration of ibogaine in rats reduces ethanol self-administration and relapse and increases GDNF expression, resulting in the activation of the GDNF pathway [227]. Importantly, the actions of ibogaine in reducing ethanol intake were localized into the VTA, and infusion of anti-GDNF neutralizing antibodies into the VTA attenuated the ibogaine-mediated decrease in ethanol self-administration [227]. Despite its attractive properties, ibogaine can induce severe side-effects such as hallucinations, whole-body tremors and ataxia that may be related to neurotoxicity in the cerebellum and dysregulation of the cardiovascular system [226].

A potential strategy to overcome these undesirable actions of ibogaine is the use of derivatives like noribogaine and 18-methoxy-coronaridin (18-MC). 18-MC was found to reduce ethanol intake in rodents, without affecting water consumption [228]. Importantly, these ibogaine-derived metabolites have no tremorigenic effects or evidence of cerebellar toxicity in animals [228].

Cabergoline, a D2-like receptor agonist, increases GDNF expression and secretion in cultured astrocytes [229]. Systemic administration of cabergoline significantly reduces operant ethanol self-administration in rats [230]. Moreover, systemic administration of cabergoline reduced both the reacquisition of operant responding for ethanol after a period of extinction and cue-induces ethanol-seeking after abstinence (which represents two different models of relapse) [230]. These results suggest that cabergoline decreases ethanol-drinking and ethanol-seeking behaviours, and that these effects are mediated by the upregulation of the GDNF pathway in the mesolimbic system. Thus, cabergoline deserves future investigations as a new alcohol pharmacotherapy [220].

**ACETALDEHYDE (ACD)**

Three main enzymatic pathways, namely alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1) and catalase, metabolize ethanol to acetaldehyde (ACD). Acetaldehyde, the first product of ethanol metabolism, plays a key role in the toxic effects of ethanol and has also been identified as a potential psychoactive drug able to induce some behavioural effects. It has been proposed that ACD is a key mediator of ethanol’s neuropharmacological and behavioural effects. Although controversial and not fully accepted, according to this hypothesis, ethanol would be a mere pro-drug whose effects are fully mediated by its first metabolite ACD [232]. Thus, a number of behavioural studies focused on ACD reinforcing properties and demonstrated that ACD self-administration is much easier to establish than ethanol self-administration [232, 233]. Thus, it was proposed that ACD is a stronger reinforcer than its parent drug ethanol. Recently, ACD was similarly shown to be a 1000-fold more potent reinforcer than ethanol when tested for self-administration into the VTA, a brain region strongly involved in ethanol reinforcing effects [234]. The reinforcing properties of ACD were also confirmed in place conditioning studies [235,236]. Notably, this place preference was stronger in rats genetically selected for their high ethanol preference [237]. Moreover ACD self-administration increased subsequent voluntary alcohol consumption in a free choice procedure [238,239].

These data support the hypothesis that ACD participates in ethanol’s psychoactive effect through its own rewarding properties. Acetaldehyde seems to have a key role as a mediator of the mesolimbic DA-stimulating effects following ethanol ingestion. In fact, in animal models both ethanol and ACD administration increase NAc DA release [240].

Consistent with in vivo reports, in vitro studies confirmed a crucial role of ACD in alcohol induced activation of midbrain DA cells. When ACD formation is prevented, ethanol ceases to enhance the spike frequency of DA neurons, suggesting that ACD mediates ethanol-induced effects on DA neuronal spontaneous activity. Further evidence is provided by the observation that ACD dose dependently produces a fast increase in DA neuronal firing activity [240-242]. Cysteine, (2R)-2-amino-3-sulfanyl-propanoic acid, is not an essential amino acid with a thiol side chain. Cysteine is efficacious to bind ACD derived from alcohol drinking to prevent the possible role of ACD in digestive tract cancer [243-245], alcoholic cardiomyopathy [246], as well as to prevent ACD chronic toxicity [247] and oxidative damage [246]. In Wistar rats, pretreated i.p. with saline or L-cysteine, followed by intragastric administration of saline, ethanol, or ACD, L-cysteine dose-dependently prevented both ethanol and ACD-induced conditioned place preference [248]. In the same animal model, pretreatment with L-cysteine reduced both acquisition and maintenance of oral ethanol self-administration behaviour [249]. In addition, pretreatment with L-cysteine reduced reinstatement of ethanol-drinking behaviour after an oral ethanol extinction [249]. These experiments show that L-cysteine reduces all phases of oral ethanol self-administration. The efficacy of L-cysteine on relapse to alcohol drinking represents an interesting pharmacological approach and could lead to new alcohol pharmacotherapies [249].

**KUDZU**

Several genetic and clinical observations indicate that a selective deficiency of aldehyde dehydrogenase 2 (ALDH-2) is often associated with reduced drinking and risk for alcoholism [250]. Subjects with ALDH-2 deficiency, such as 15 to 40% of Southeast Asians, have an inactivation of ALDH-2 due to an E487K mutation [251]. These individuals, after taking alcohol, experience unpleasant symptoms due to the accumulation of ACD [250]. Acetaldehyde is a product of hepatic alcohol dehydrogenase (ADH) and is metabolized to acetate by cytoplasmic ALDH-1 and mitochondrial ALDH-2.

It was suggested that administration of drugs that inhibit ALDH activity and consequently, induce elevations in ACD, discourage alcoholics to continue drinking. It should be noted that although low levels of brain ACD may be reinforcing in the VTA, high systemic concentrations of ACD are aversive, producing nausea, diaphoresis and tachycardia. The most studied drug with this mechanism of action is the FDA-approved medication disulfiram (see [10]), a nonspecific toxic and irreversible inhibitor of both mitochondrial ALDH-2 and cytoplasmic ALDH-1 [252]. However, disulfiram may have non-specific toxicity caused by chelating metals and reacting with sulfhydryl groups to inactivate diverse proteins and other enzymes, in addition to ALDH-1 and ALDH-2. Thus, research has focused on identifying more selective inhibitors of mitochondrial ALDH-2. Kudzu and kudzu extracts have been identified as such compounds. Kudzu is a perennial leguminous vine of the genus *Pueraria lobata* native to eastern Asia [253]. *Pueraria*, daidzin, and daidzein are three of the major isoflavonoid compounds isolated from the extract of *Pueraria lobata* [253]. The anticraving and “antidrunkeness” effects of extracts of *Pueraria lobata* have been known to traditional Chinese Medicine for centuries, but its true efficacy and the mechanism(s) of action of its active principle(s) were unproven.

Daidzin was first shown to suppress ethanol intake in ethanol preferring Syrian golden hamsters [254]. Subsequently, it was found that all three isoflavonoid compounds were effective in suppressing voluntary alcohol consumption in alcohol-prefering (P) rats. In particular, daidzein, daidzin, and puerarin, orally administered to P rats at a dose of 100 mg/kg/day, decrease ethanol intake by 75%, 50%, and 40%, respectively [255]. The efficacy of *Pueraria lobata* extracts in reducing voluntary alcohol consumption has been confirmed in all ethanol-drinking animals tested to date [256-260]. The most recent preclinical study tested the efficacy of a highly selective reversible ALDH-2 inhibitor, CVT-10216, in models of moderate and high alcohol drinking rats [261]. Results of this
study showed that treatment with CVT-10216 reduces heavy drinking in rodents, suppresses alcohol self-administration, deprivation-induced drinking and cue-induced reinstatement of alcohol-seeking, an animal model of relapse. The authors suggested that CVT-10216 may prevent alcohol-induced increase in NAAD.

A study by Xie and colleagues [262] showed that i.p. administration of dazidin reduces intoxication induced by anaeasthetic doses of alcohol in rats. Notably, the effects of dazidin in shortening alcohol-induced sleep time, a reliable measure of alcohol intoxication, was found after intragastric, but not i.p. infusion of alcohol, suggesting that the effects of dazidin are also due to a slowdown of gastric emptying, which would expose alcohol for a longer time to first-pass metabolism [263]. Some effects in reducing alcohol withdrawal symptoms were also observed for dazidin and pu-erarin [259,260,264]. In particular, Overstreet and colleagues showed that the extract of kudzu NIH-031G (puerarin) was effective in counteracting anxiogenic effects associated with alcohol withdrawal in rats exposed chronically to alcohol, suggesting an underlying mechanism of antagonism at the benzodiazepine receptors [264].

In humans, a randomized double-blind controlled trial evaluated the efficacy of kudzu root extracts in influencing drinking habits of veterans who entered a substance abuse treatment program [265]. Alcohol dependent patients were randomly assigned to receive kudzu root extracts 1.2 g twice daily (21 patients) or a matching placebo (17 patients). In this small sample, kudzu root was no better than placebo in reducing alcohol craving, or in promoting abstinence [265]. A subsequent clinical trial was conducted to evaluate the efficacy of a kudzu extract in heavy drinkers treated with either a kudzu extract or placebo for 7 days [266]. Participants had then the opportunity to drink their preferred brand of beer while in a laboratory setting. Results of this study showed a significant reduction in the number of beers consumed in one week of treatment, as well as an increase in the number of sips, an increase in the time to consume each beer and a decrease in the volume of each sip. There were no changes in the urge to drink alcohol, suggesting that kudzu probably does not act blocking alcohol’s effects but instead prolonging or enhancing the acute effects of the first drink [266].

NICOTINIC AND MUSCARINIC ACETYLCHOLINE RECEPTORS (nAChRs AND mAChRs)

The central cholinergic system has been implicated in the development of alcohol and drug addictions. The receptors of the cholinergic system are divided into muscarinic acetylcholine receptors (mAChRs) and nicotinic acetylcholine receptors (nAChRs). The potential role of nAChRs in the treatment of alcohol and drug addictions has been more extensively studied than that of mAChRs [267].

Mesocorticolimbic DA neurons are known to contain central nAChRs that modulate the accumal release of DA [268]. Alcohol-induced stimulation of the mesolimbic DA system may involve central nAChRs, suggesting their possible involvement in the initiation phase of alcohol consumption [269]. Other experiments also demonstrated an involvement of these receptors in the mechanisms of maintenance of alcohol consumption and relapse to alcohol use [269]. Blomqvist and Ericson have extensively studied the involvement of nAChRs in the mechanisms of alcoholism and found that ethanol activates the mesocorticlimbic DA system via both direct and indirect stimulation of central nAChRs NAAC [270,271]. Moreover, mecamylamine, a blood brain barrier penetrating nAChR antagonist, completely antagonizes both of these effects [272]. On the basis of these observations, the effects of two nAChR antagonists, mecamylamine and hexamethonium, as well as the effects of subchronic nicotine treatment on voluntary ethanol consumption were studied in ethanol low-, medium- or high-prefering Wistar rats [270]. Mecamylamine but not hexamethonium reduced ethanol intake in high-prefering rats, while subchronic nicotine treatment increased ethanol consumption in rats with a medium preference for alcohol. Neither mecamylamine nor hexamethonium reduced ethanol preference. On the basis of these results, the authors suggested that antagonists at nAChRs could be useful compounds in the treatment of alcoholic subjects [270]. A subsequent study by the same research group provided further evidence on the possible role of mecamylamine in the treatment of alcoholism [273] as ethanol intake and preference in Wistar rats were markedly reduced and DA levels were unaltered with respect to pre-drug baseline levels [273]. In a more recent study, Farook and colleagues [274] using alcohol-prefering C57BL/6J mice reported that there was a reduced consumption of alcohol in daily and intermittent mecamylamine-treated (intermittent) mice than in control mice.

Twelve different nAChR subunits (α2-α10 and β2-β4) have been identified and these subunits can constitute a multitude of pentameric nAChRs with different molecular configurations. These nAChRs are expressed in varying patterns and densities throughout the brain, e.g. the mRNAs for the nAChR subunits α3-α7 and β2-β3 have been found highly expressed within the VTA [275]. Moreover, variations in the nAChR subunit composition may influence the sensitivity to ethanol. In order to examine the role of different nAChR subunits in operant ethanol self-administration and in ethanol drinking relapse following a 10-day period of ethanol deprivation, Kuzmin and colleagues [275] compared the nicotinic subunit-specific antagonist α-conotoxin MIII (αCtxMIII, α3 [β2, β3, α6] with systemic mecamylamine, dihydro- β-erythroidine (DHβE, α4 β2) and methyllycaconitine (MLA, α7). αCtxMIII was administered directly into VTA, whereas mecamylamine, DHβE and MLA were administered systemically. Results of the study showed that αCtxMIII reduced operant ethanol self-administration and blocked the deprivation-induced relapse-like ethanol consumption; mecamylamine reduced operant ethanol self-administration and inhibited the deprivation-induced increase in alcohol consumption; DHβE inhibited ethanol intake only at a higher dose; MLA failed to block self-administration of ethanol and relapse-like drinking after deprivation. These results suggested that modulation of central effects of ethanol are mediated mainly by α3β2 and/or β3 subunits of nAChRs. Therefore, ligands for these subunits might be of greater interest for alcohol pharmacotherapies [275].

 Lobeline is a naturally occurring alkaloid obtained from the plant Lobelia inflata, that binds to nAChRs with high affinity, displaying both agonist and antagonist activity. In a recent study, the effects of repeated administration (continuous or recurring for 5 days) of the partial nicotinic agonist lobeline on alcohol consumption and alcohol preference were investigated in a high alcohol preferring strain of mice [276]. Lobeline attenuated alcohol consumption and preference during both the repeated-recurring and continuous administration phases, probably by activating both high (α4β2) and low affinity (α7) central nAChRs [276]. The effects of lobeline and of the nAChR partial agonist cytisine on ethanol drinking were then investigated by Bell and colleagues [276]. At high doses both nAChR ligands independently suppressed ethanol intake. Specifically, cytisine suppressed ethanol intake, by attenuating α4β2 nAChR function and lobeline suppressed ethanol intake, by acting as a nonselective competitive antagonist at αβ2 and α3β2 nAChRs. Practically, both cytisine and lobeline act as functional antagonists at nAChRs, which in turn lead to reduce alcohol induced DA release in the NAc and consequently, to attenuate the reinforcing properties of ethanol.

Varenicline, a cytisine derivative, is a partial agonist at the α4β2 nAChRs and has been approved for marketing in the U.S. and in more than 30 countries worldwide as an aid for smoking cessation [277]. Varenicline, at higher concentrations, can interact with other nAChRs subtypes, in addition to α4β2, including the α7 homomeric receptor subtype. A role of α7 nAChRs in modulation of ethanol response has been hypothesized, although a recent study using mice with a constitutive knockout of either the α7 or β2 subunit found that neither subunit is absolutely required for the
effect of varenicline on ethanol intake [278]. Steensland and colleagues [279] investigated the role of varenicline in the modulation of alcohol consumption and alcohol seeking using three different animal models of drinking. Acute administration of varenicline, in doses reported to reduce nicotine reward, selectively reduced ethanol but not sucrose seeking and decreased voluntary ethanol consumption in animals chronically exposed to ethanol for two months before varenicline treatment. Moreover, chronic varenicline administration reduced ethanol consumption, which did not result in a rebound increase in ethanol intake when varenicline was discontinued. Furthermore, varenicline appears to ameliorate ethanol-induced cognitive deficits, in particular deficits in acquisition of contextual and cued associative learning [280].

Few studies investigating the efficacy of nAChRs ligands in the treatment of alcoholism have been conducted in humans. The first clinical study by Blomqvist and co-workers [281] investigated the effects of mecamylamine on subjective responses of healthy subjects to a moderate dose of alcohol. Results of the study showed that mecamylamine, as compared to placebo, reduced the stimulant and pleasurable effects of an acute administration of alcohol during the ascending limb of the blood alcohol curve, probably by modifying both the pharmacokinetic profile of alcohol and the rewarding effects. Chi and de Wit [282] replicated and extended this study using a double-blind design. As compared to placebo, mecamylamine reduced subjective euphoria after alcohol intake and desire to consume more alcohol. However, a more recent study with mecamylamine did not confirm these observations [283]. To date only one human study using varenicline for alcoholism has been published [284]. This study was a double-blind, placebo-controlled trial in which the effect of varenicline (2 mg/day vs. placebo) on alcohol self-administration was investigated in 20 non-alcohol-dependent heavy drinkers who were also daily smokers. After seven days of medication pre-treatment, subjects were first administered a priming dose of alcohol (3g/kg) and subjective and physiologic responses were assessed. The participants were exposed to a 2-hour drinking period during which they were permitted to drink up to eight alcoholic drinks or to receive monetary reinforcement for each drink not consumed. Results showed that varenicline significantly reduced the number of drinks consumed compared to placebo and increased the likelihood of abstaining from any drinking during the self-administration period. Furthermore, following the priming drink, varenicline attenuated alcohol craving and reduced subjective reinforcing alcohol effects, without significant side effects.

These promising results have aroused interest in respect of varenicline as a new medication target for alcohol use disorders. In fact, there are several ongoing clinical and human laboratory trials designed to investigate both the effects of varenicline on heavy drinking and the effectiveness in the treatment of AD (e.g. NCT00846859, NCT01071187, NCT00695500).

Muscarnic acetylcholine receptors (mAChRs) are predominantly presynaptic autoreceptors, being responsible for acetylcholine-mediated inhibition of adenyl cyclase activity. mAChRs primarily provide negative feedback on acetylcholine release from cholinergic terminals [285] and are involved in many brain function, including attention, learning, memory and cognition [286]. Several studies showed an association between the muscarinic acetylcholine M2 receptor (CHRM2) gene and AD [286,287]. In particular, one haplotype block within the 5'-UTR of CHRM2 may be more important for the development of alcoholism than other regions [287]. Thus, an involvement of mAChRs in the neurobiological mechanisms of alcoholism could be hypothesized. However, at present there are no human or animal studies that have investigated mAChRs as a new potential target for the treatment of AD.

ACETYLCOLINESTERASE INHIBITORS

A possible role of acetylcholinesterase inhibitors for alcoholism has been hypothesized. In fact, cholinesterase inhibitors act on the breakdown of the neurotransmitter acetylcholine and prolong acetylcholine retention time in the synaptic cleft and therefore, the efficiency of cholinergic neurotransmission at both mAChRs and nAChRs [288]. Galantamine, an alkaloid of many plants of the Amaryllidaceae family and of various species of narcissus, is a reversible acetylcholinesterase inhibitor which also acts as an allosteric potentiating ligand at neuronal nAChRs, especially at the α7 and α4β2 nAChRs, but not at mAChRs [289]. Opitz [290] first described a role of galantamine in reducing ethanol preference in an animal model of AD. After oral administration of galantamine at doses of 5 or 10 mg/kg, in the following 1-2 h exposition time both alcohol preference and ethanol intake were reduced significantly. Galantamine reduced alcohol intake without decreasing food consumption and total fluid intake. Subsequently, galantamine has also shown to improve the speed of learning, short-term memory and spatial orientation in rats after prolonged (16 weeks) alcohol intake [291]. This last observation is consistent with the hypothesis that prolonged alcohol intake could lead to an acetylcholine deficit and to an impairment of many brain functions.

More recently, the effects of galantamine on voluntary ethanol consumption were investigated in female Alko alcohol rats, in combination with desoxypygeanine, an alkaloid derived from Peganum harmala. In this experiment, desoxypygeanine, initially administered reduced ethanol intake and ethanol preference and these effects were dose-dependent, at a dose ranged between 10 and 30 mg/kg body weight. In this preclinical study, the combination of desoxypygeanine and galantamine in doses which were ineffective when administered alone, caused a significant decrease of ethanol preference in rats, suggesting an addictive effect [292].

Based on the preclinical studies, Mann and colleagues [293] investigated the efficacy of galantamine in prolonging abstinence in recently detoxified alcoholics. A 24-week, randomized, placebo-controlled, multicenter clinical trial involving 149 recently detoxified alcohol dependent patients was conducted. Galantamine at the dose of 25 mg/d or placebo were administered by a patch that was renewed every morning during the first 12 weeks (treatment phase), then no patch was applied during the successive 12-week period (follow-up phase). Patients also received a low-intensity psychosocial standard therapy at each visit. Results of the study showed that galantamine, compared to placebo, did not extend the time to first severe relapse. Moreover, at the end of the 12-week treatment period, significantly fewer patients assigned to galantamine remained abstinent (20%) compared to placebo group (41%). The same results were observed at the end of the follow-up period (week 24). The contradictory findings, between preclinical and clinical observations, could be explained by the different way galantamine was administered. Compared with oral administration, the patch offers a very different pharmacokinetic profile and probably, the applied dose of 25 mg/d was not sufficient to achieve effective plasma levels of the drug. In fact, the average plasma concentration of galantamine was 7.1 ± 9.1 ng/ml, lower than the expected 16.7 ± 9.35 ng/ml [293]. Another explanation, provided by the authors, is related to the rapid eye movement (REM). REM pressure during sleep is dependent on the central cholinergic system and rises with increased cholinergic activity [294]. In turn, the increased REM sleep induced by galantamine in alcohol dependent patients after alcohol withdrawal represents a robust predictor of vulnerability to relapse [294]. Therefore, it might be possible that an increased REM induced by galantamine induced the recently detoxified alcoholics to relapse [293]. Nevertheless, further studies are needed to explore if galantamine represents a potential treatment for AD.
GLYCYNE REUPTAKE INHIBITORS

Ethanol administration is known to increase DA output in the NAc of the rats [295] as well as in humans [2], and this increase has been hypothesized to be of importance for the development of AD [296]. The mechanisms by which DA participates in alcohol-seeking behaviour and how the mesolimbic DA activity is regulated have been extensively investigated. Recently, glycine and strychnine-sensitive glycine receptors (GlyRs) generated interest in the field of AD, considering their involvement in the DA activity.

Glycine is the major inhibitory neurotransmitter in the spinal cord and brain stem and it has been implicated in controlling neuronal excitability and psychotic symptoms [297]. Glycine receptors have also been identified in the CNS and they are expressed on both dopaminergic and non-dopaminergic cells of the VTA [298]. Glycine activates GlyRs and determines a reduction of inhibitory GABAergic inputs to VTA dopaminergic neurons, producing an increase in accumbal DA release [298]. In vitro studies showed that ethanol potentiates glycine receptor function [299,300]. Successively, Mølander and Soderpalm [301,302] demonstrated that GlyRs are expressed in the rat NAc and that activation of these receptors might constitute a priming mechanism for ethanol-induced enhancement of accumbal DA activity. In particular, a local perfusion of strychnine, a GlyR antagonist, decreases accumbal DA levels per se and completely prevents the increase of accumbal DA levels after both local and systemic ethanol administration. Instead, accumbal perfusion of the GlyR agonist glycine increases DA levels in a subpopulation of rats and prevents the ethanol-induced increase after local but not systemic ethanol in all animals [302]. The same authors also investigated the role of accumbal GlyRs in the regulation of voluntary ethanol intake in the rats and found that glycine and strychnine altered extracellular DA levels in the NAc, probably via GlyR stimulation and blockade, respectively [303]. Concomitantly, strychnine and glycine reciprocally altered ethanol consumption in ethanol-prefering rats, respectively decreasing and increasing ethanol intake [303]. Thus, results of these experiments suggest that GlyRs agonists and/or antagonists could represent interesting new ways to treat alcohol dependence.

Other research has focused on the possible role of glycine transporter (GlyT) inhibitors in voluntary alcohol consumption. GlyT proteins are involved in the reuptake of glycine into presynaptic nerve terminals and glial processes, allowing the return of extracellular levels of glycine to basal values. There are two known types of GlyT proteins, the GlyT1 and the GlyT2 located on glia cells and glycinerergic neurons, respectively. The GlyT1 catalyses the removal of glycine from the synaptic cleft and the GlyT2 is involved in the reuptake and reloading of glycine into synaptic vesicles [304]. Mølander and colleagues [304] investigated the role of a selective glycine reuptake inhibitor (Org 25935) in decreasing alcohol intake and preference in rats. Org 25935 easily passes the blood-brain barrier and acts mainly on the GlyT1 protein. Ge et al. [305] administered Org 25935 via i.p. to male Wistar rats with an ethanol preference > 60% (during continuous access to a bottle of alcohol, 6% v/v, and a bottle of water) and to rats with an ethanol preference < 60%. Org 25935 was effective in reducing alcohol intake in both populations of rats. This effect was dose-dependent, developed gradually and was sustained for up to 40 days. Food intake was also transiently reduced. The mechanism underlying the effects of Org 25935 in reducing alcohol intake is probably represented by an increase of extracellular glycine levels and a subsequent modulatory effect on brain GlyRs. A more recent study investigated the DA output in the NAc after systemic administration of Org 25935 and ethanol [306], that suggests the involvement of the glycine/GlyR system in modulating basal accumbal DA levels and the ethanol-induced elevations of DA in the NAc. Based on these studies, it was supposed that the anti-alcohol effect of Org 25935 is due to i) a substitution mechanism by stimulation of GlyRs and elevation of DA levels; and ii) antagonism mechanism by prevention of further GlyR activation and DA elevation by ethanol.

Based on this first preclinical evidence, a double-blind, placebo-controlled clinical trial is currently investigating the safety, tolerability and efficacy of Org 25935 on alcohol-dependent individuals (NCT00764660).

ALPHA-ADRENERGIC RECEPTOR

Norepinephrine in the limbic system is important to emotional learning and may be involved in the neuroadaptations that lead to the development of dependence. In the amygdala, increased norepinephrine (NE) activity leads to creation of stronger and longer-lasting memories [307]. NE depletion produced by blockade of the synthetic pathway attenuates ethanol-self administration in rats [308]. Ligands targeting different receptor subtypes of the NE system have been most extensively studied for reducing certain aspects of ethanol withdrawal symptomology. In particular, it has been shown that β-adrenergic antagonists and α2-noradrenergic receptor agonists appear to be efficacious in reducing withdrawal symptoms and α1-noradrenergic receptor antagonists in reducing locomotor hyperactivity produced by ethanol withdrawal [309].

Though early work suggested that the NE reuptake inhibitor desipramine prolonged the time to relapse in depressed alcoholics [310,311], research on alcohol pharmacotherapy has been slow to focus on the NE system. Recently, this interest has been renewed. Initially, research focused on the α2-adrenoceptor antagonist yohimbine, while more recent research is focusing on the prototype α1-blocker prazosin.

Yohimbine, an α2-adrenoceptor antagonist which acts by increasing norepinephrine cell firing and norepinephrine release in terminal areas, was proposed as an effective drug in the treatment of AD [312]. In this study, injections of the α2-adrenergic receptor antagonists, yohimbine and methoxyprazoxan, to rats decreased intake of alcoholic beverage and increased intake of water. Furthermore, yohimbine alone persistently reduced intake of alcohol with daily administration [312]. However, subsequent studies did not confirm these observations. Indeed, it was shown an effect of yohimbine in reinstating alcohol seeking both in rats and in humans probably by inducing anxiety- and stress-like response and acting as a pharmacological stressor [313].

Prazosin, an α1-noradrenergic receptor antagonist, has aroused interest as effective drug in reducing alcohol intake. Prazosin was evaluated for the ability to modulate operant ethanol self-administration in non-dependent and ethanol-dependent animals during acute withdrawal [309]. In this study, prazosin at different dosages (0.0, 0.25, 1, 1.5 and 2 mg/kg) was administered to ethanol dependent and control rats during acute withdrawal. Prazosin reduced self-administration of ethanol in both non-dependent and ethanol-dependent rats during acute withdrawal. However, prazosin was more potent in ethanol-dependent animals, suggesting an increase in the sensitivity to prazosin in dependent animals due to alterations in the norepinephrine system during chronic exposure to ethanol. Then, Rasmussen and colleagues [314] tested the efficacy of prazosin in suppressing alcohol drinking in rats selectively bred for alcohol preference (P line). Adult male P rats were given open access to food and water and scheduled access to a 15% (v/v) alcohol solution for 2 hours daily. Prazosin at different doses (0, 0.5, 1.0, 1.5, or 2.0 mg/kg body weight) was injected i.p., once a day prior to onset of the daily 2-bottle choice (alcohol vs. water), for 3 weeks. Prazosin significantly reduced alcohol intake during the initial 2 daily administrations, and this reduction of alcohol intake was maintained in the subsequent more prolonged trial and was not dependent upon drug-induced motor impairment.

Based on these promising preclinical results, Simpson and colleagues [315] tested the effectiveness of prazosin in reducing drinking and craving for alcohol among AD patients. Twenty-four alco-
hol-dependent patients were randomized to receive either prazosin or placebo in a 6-week, double-blind pilot study. Medication was titrated to a target dose of 4 mg QAM, 4 mg QPM, and 8 mg QHS by the end of week 2. Results of the study showed fewer drinking days and drinks per week in the prazosin group with respect to the placebo group during the final 3 weeks of the study. With regard to craving, although the entire sample on average reported significantly decreased craving from baseline to the final assessment, the prazosin group did not report significantly less craving than the placebo group. No significant side-effects were reported, although one of the prazosin-treated participants experienced syncope, requiring a dose reduction. At present, large controlled studies are in progress to further investigate the role of prazosin in AD (e.g. NCT007062710).

CALCIUM-ACTIVATED-POTASSIUM (CAK) CHANNELS

In CNS neurons, calcium-activated-potassium (CAK) channels modulate neuronal activity by controlling intrinsic excitability, tonic firing frequency, spike frequency adaptations, and action potential repolarization. These channels are activated by elevations in intracellular Ca2+ that occurs during single or trains of action potentials, and their activation is thought to contribute to the prolonged afterhyperpolarization that follows an action potential.

CAK channels are defined by two phenotypic characteristics: their pores conduct K+ rather selectively over other monovalent ions, and their steady-state activity is enhanced by increases in intracellular calcium. Based on their K+ permeability, three types of CAK channels have been recognized and they demonstrate heterogeneous pharmacological and kinetic properties: small (SK), intermediate (IK) and large conductance (BK or MaxiK) [316,317]. SK and BK have been more extensively studied in alcohol dependence.

SK channels underlie the medium phase of the hyperpolarization and are important for shaping postsynaptic responses and controlling intrinsic excitability, dendritic integration, and pacemaker firing.

The spontaneous action potential of VTA neurons is determined by a number of voltage and Ca2+-dependent currents, which involve SK channels [318]. Since ethanol directly excites DA VTA neurons [319], a recent study addressed the possibility that repeated ethanol exposure and withdrawal leads to altered firing properties of VTA DA neurons through changes in SK channel function [320]. Specifically, Hopf and colleagues [320] used ex vivo brain slice electrophysiology to characterize whether pacemaker firing and NMDA-induced burst firing in VTA neurons were altered after 5 days of intermittent ethanol exposure and 7 days of withdrawal, and what channels might be altered functionally to explain any observed firing differences. This study demonstrated that repeated ethanol and withdrawal leads to a significant reduction in VTA SK currents [320]. Thus, SK channels are a critical component of the VTA neuronal membrane conductance and play an important role in DA VTA neurons, i.e. altering the responsiveness of these neurons to external stimulation, either by endogenous agents, like neurotransmitters, or by exogenous agents, like alcohol.

Other studies showed that the excitatory effects of ethanol can be modulated by a number of agents that block SK channels or affect Ca2+ levels and release [318]. Moreover, manipulation of Ca2+ levels and release can result in reduction of overall Ca2+ and, as a direct antagonism of SK, these manipulations can increase the excitatory action of ethanol [321]. Finally, a recent study has shown an association between reduced small-conductance calcium-activated potassium channel (SK) currents and increased firing in the NAc core after protracted abstinence from alcohol but not in cocaine self-administration [322]. In conclusion, consistent with evidence of an interaction between ethanol and SK currents in reward neurons of the VTA, SK channels may represent a novel target to treat AD.

The BK channels are largely expressed in the brain and contribute to the fast phase of the AHP. These channels play an essential role in many aspects of neuronal physiology, including neurotransmitter release, action potential patterning and dendritic excitability. In addition, a number of studies have also implicated an important role for BK channels in ethanol tolerance and adaptive plasticity [317-323].

The BK channel is composed of a primary protein, containing a pore that acts as a conduit for K+ ions. This primary protein is often combined with a variety of auxiliary proteins (b1-b4) that modify the function of the channel, including alcohol sensitivity. Compartment-specific expression of b subunits of the BK channels has been proposed to explain differences in BK-related alcohol sensitivity between the somatic, dendritic and terminal compartments of neurons [324]. One of these auxiliary proteins, known as b4, plays a key role in acute alcohol tolerance. In fact, an experiment with mice knocked out for the gene encoding the b4 subunit [325] showed that the b4 subunit inhibits acute alcohol tolerance and the presence of acute tolerance is an indicator of alcohol consumption.

There is also evidence that lipid composition of biological membranes is altered differently by acute and chronic alcohol exposure as experiments with BK channels provide evidence of the powerful effects that lipid environment can have in both the immediate and adaptive response to alcohol, considering that BK might move into and out of lipid rafts, thereby changing both the acute response and adaptation to alcohol [326,327].

VOLTAGE-GATED CALCIUM CHANNELS

Voltage-gated calcium channels (VGCCs) are multimeric protein complexes that mediate Ca2+ entry into neurons in response to changes in membrane potential and regulate neuronal excitability, neurotransmitter release, and gene expression. They are classified according to their electrophysiological and pharmacological properties [328]. High voltage-activated N-type calcium channels are exclusively expressed in the nervous system, where they contribute to neurotransmitter release at a subset of central and peripheral nerve terminals [329]. Low voltage-activated T-type calcium channels are biophysically distinct from N-type channels and contribute to rhythmic firing and bursting behaviours related to processes such as sleep and epileptiform activity [330].

Acute ethanol exposure inhibits N-type calcium channels, and chronic ethanol exposure increases N-type channel function and density. Ethanol appears to have complex effects on T-type currents. In slices from the lateral geniculate nucleus, low concentrations of ethanol potentiate T-type currents, whereas higher ethanol concentrations inhibit T-type currents [331]. The principal defined role for N-type calcium channels is the regulation of evoked neurotransmitter release [332].

Newton et al. [333] chose a genetic approach by studying ethanol responses in mice that carry a null mutation in the calcium channel subunit Cav2.2 and therefore lack functional N-type calcium channels. In these animals, voluntary ethanol consumption is reduced and place preference is developed only at a low dose of ethanol. The hypnic effects of ethanol are also substantially diminished, whereas ethanol-induced ataxia is mildly increased. These results demonstrate that N-type calcium channels modulate acute responses to ethanol and are important mediators of ethanol reward and preference [333].

The same group tested the effect of a novel mixed inhibitor of N-type and T-type calcium channels, 1-[(6,6-bis(4-fluorophenyl)hexyl)-4-(3,4,5- trimethoxybenzyl) piperazine (NP078585) in animal model of ethanol intoxication, reinforcement, reward, and reinstatement. The results of these studies showed that NP078585 reduced the acute effects of ethanol and abolished the expression of ethanol conditioned place preference in mice [334] supporting the
hypothesis that N-type calcium channels contribute to ethanol intoxication, reinforcement, reward, and reinstatement.

Considering that N-type calcium channel inhibitors are being developed for clinical use, these data show the possibility that AD individuals treated with N-type or mixed N- and T-type calcium channel inhibitors may become less motivated to experience the effects of ethanol.

SIGMA-RECEPTORS (SigRs)

Sigma receptors (SigRs) were originally called as sigma/opioid receptors because the effect of the prototypic sigma/opioid receptor ligand, N-allylnormetazocine, was reported to be antagonized by the opioid antagonist naloxone [335]. At least two subtypes of sigma receptors, SigR1 and SigR2, are known.

Because SigR1s are involved in regulating dopaminergic, NMDA and glutamatergic neurotransmission in limbic areas, including the NAc and the VTA a role of SigR1 in development of AD has been hypothesized. Miyatake and colleagues [336] performed a functional analysis of polymorphisms in the SigR1 and a case-control study, involving 307 alcoholic and 302 control subjects. These investigators not only identified the novel T-485A polymorphism, but also found that the transcriptional activity of the A-485 allele and the TT-241-240 allele was significantly reduced compared with that of the T-485 allele and the GC-241-240 allele. Moreover, the frequencies of the A-485 allele and the TT-241-240/Prc2 haplotype were significantly higher in control subjects compared with alcoholic subjects, suggesting a possible protective role for the development of alcoholism [336].

More recently, a role of SigR antagonists in modulating ethanol’s actions has been proposed. A recent study investigated the effects of subcutaneous treatment with the potent, selective SigR1 antagonist BD-1063 on operant ethanol self-administration in two models of excessive drinking (Sardinian alcohol-prefering (sP) rats and ethanol-dependent Wistar rats), compared to non-dependent Wistar controls [337]. BD-1063 dose-dependently and selectively reduced oral ethanol self-administration in sP rats and in ethanol dependent Wistar rat. Moreover, BD-1063 reduced the motivation of sP rats to work to obtain alcohol. A significant decrease of SigR1 mRNA expression in the NAc was found in both ethanol-naive sP rats and ethanol-dependent Wistar rats with respect to ethanol-naive control Wistar rats, suggesting a role of SigR1s in the modulation of excessive ethanol intake and reinforcement and a role of SigR1s antagonists as potential target for medication development for treatment of alcoholism [337]. Further confirming preclinical studies and exploratory human studies are needed to confirm this initial evidence.

MELANOCYTE STIMULATING HORMONE (MSH)

A role for melanocortins (MC) peptides in neurobiological responses to drugs of abuse has been established. The MC system is composed of peptides that are cleaved from the polypeptide precursor, proopiomelanocortin (POMC), which is also the precursor for β-endorphin. These peptides include adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-MSH, and γ-MSH [338]. The production of these peptides occurs in the neurons within the hypothalamic arcuate nucleus, the nucleus of the solitary tract, and the medulla. At least five subtypes of MC peptides receptors, including MC4R, have been identified [338], which have been implicated in most behavioural effects including neurobiology of depression [339].

An involvement of MC signalling in AD has been suggested. In order to investigate the involvement of the MCs in AD, Ploj and colleagues [340] analysed the effects of the MC4R antagonist HS014 (1 nmol/rat), and the non-selective MC-receptor agonist MTII (1 nmol/rat), administered i.c.v., on ethanol intake in alcohol-prefering AA rats. MTII caused a reduction in ethanol intake and ethanol preference, whereas HS014 had no any effect. Simultaneously, MTII and HS014 altered opioid peptide levels in several brain areas and in the pituitary gland of alcohol-prefering AA rats the rats.

Navarro and colleagues [341] extended the previous findings [340] by showing that both central and peripheral administration of MTII reduces ethanol drinking. Subsequently, the same group demonstrated that an i.c.v. infusion of the highly selective MC4R agonist cyclo (NH-CH2-CH2-CO-His-d-Phe-Arg-Trp-Glu-NH2) at doses of 1.0 or 3.0 microg significantly reduced ethanol drinking in rats [342]. In a more recent study, the role of α-MSH in mediating the acute and chronic effects of ethanol and withdrawal-related depression was investigated in rats [338]. Results of the study showed that α-MSH may be closely involved in mediating the actions of ethanol and that the nature of interaction may be dictated by the protocol of ethanol treatment. Central administration of α-MSH (100 ng/rat, i.c.v.) was found to suppress the anti-immobility effect of acute ethanol, while HS014 (0.01 ng/rat, i.c.v.) was found to enhance. Chronic ethanol exposure resulted in increased immobility time, while further augmentation in ethanol withdrawal. However, concomitant HS014 (0.01 ng/rat, i.c.v.) treatment prevented tolerance as well as attenuated enhanced immobility in ethanol-withdrawn rats. In brief, while acutely administered ethanol causes antidepressant-like effect and may trigger antagonistic homeostatic response from the α-MSH, chronic ethanol treatment results in up-regulation of the entire α-MSH system that may contribute to the tolerance.

The present data support the hypothesis that the endogenous MC system modulates neurobiological responses to ethanol. Thus, compounds that target MCRs may have therapeutic value in the treatment of excessive ethanol consumption and/or the symptoms associated with ethanol withdrawal.

VASOPRESSIN

A growing body of evidence suggests that vasopressinergic neuronal activity in the amygdala and PVN of the hypothalamus represents an important element in the neurobiology of stress-related behaviours. In fact, acute stress increases extracellular levels of arginine vasopressin (AVP) in the rat amygdala and hypothalamus [343,344], and activation of AVP-receptors modulates anxiogenic and depressive behaviours in rats [344]. Vasopressin binds to three different G protein-coupled receptor subtypes: V1a, V1b, and V2. The V2 receptor is expressed almost exclusively in the kidney. The V1a and V1b receptors are localized in the brain, and the distribution of vasopressin receptor binding is prominent in the rat extended amygdala, with high concentrations in the lateral and supracapsular bed nucleus of the stria terminalis, the central nucleus of the amygdala, and the shell of the NAc [345].

Prolonged or chronic ethanol exposure decreases vasopressin-like immunoreactivity in the hypothalamus and the bed nucleus of the stria terminalis projection to the lateral septum [346]. A selective V1b receptor antagonist dose-dependently blocks the increase in ethanol self-administration during withdrawal in dependent rats but has no effect in nondependent animals [347]. To date, few studies have explored the motivational effects of vasopressin antagonists in animal models of dependence or stress-induced reinstatement with other drugs of abuse. However, the literature suggesting that V1b antagonists have anxiolytic like profiles and that vasopressin and its receptors are highly expressed in the extended amygdala lead to hypothesize that the vasopressin system may have a role in the increased alcohol intake associated with dependence [347].

LUTEINIZING HORMONE (LH)

Hypothalamic luteinizing hormone releasing hormone (LHRH) neurons are projected to some of the brain regions implicated in the regulation of behaviour including the NAc and VTA. This may explain why LHRH analogues may exhibit a variety of effects such
as antidepressant, anti-anxiety, analgesic, anticonvulsant, catalepsy, drug discrimination learning, and inhibition of condition avoidance response [348]. LHHR also modulates the activity of serotonin, glutamate, DA and opioids. A recent study showed that the LHHR agonist leuprolide exhibits an anticompulsive-like effect, and also mediates the anticompulsive effect of fluoxetine [349]. In view of the evidence that ethanol administration to rats reduces hypothalamic content of LHHR, suppresses LHHR secretion, reduces LHHR mRNA levels and inhibits LHHR binding to its receptor, it was speculated that ethanol dependence and withdrawal syndrome may be related to changes in the LHHR system [348]. In this connection, the effects of the LHHR agonist leuprolide on ethanol dependence were investigated in animals [348]. Since leuprolide modulates sex hormones levels, which are reported to influence behaviour, the studies were also carried out in castrated state.

The results of this study suggested that acute administration of leuprolide attenuated the expression of ethanol withdrawal behaviour, whereas on chronic administration, it attenuated the development of AD, representing a potential new target.

STEROID BIOSYNTHESIS

Peripheral neuroactive steroids can act in the CNS as allosteric modulators of neurotransmitter receptors, such as GABA, NMDA, and sigma receptors. For instance, the progesterone metabolites allopregnanolone (ALLO), Tetrahydroleoxytococorticoesterone (THDOC), and pregnanolone act as potent positive allosteric modulators at the GABA-A receptor [350]. Endogenous steroids have anxiolytic, anticonvulsant, analgesic and hypnotic properties, and also attenuate stress responses mediated by the HPA axis [351].

Some studies have shown that ethanol intake produces an elevation in both brain ALLO concentrations [352] and in plasma ALLO levels [353], in animals and humans, respectively. Furthermore, exogenous ALLO application stimulated limited-access ethanol drinking in male mice [354]. Moreover, administration of the 5a-reductase inhibitor finasteride (FIN), which decreases endogenous levels of ALLO, significantly altered ethanol drinking. In particular, subchronic FIN treatment (7 days) significantly attenuated limited access ethanol preference drinking in male C57BL/6J mice [355]. Similar modifications were also observed in humans, where acute administration of FIN changes in both subjective states and neuroactive steroid concentration [356]. The observed correlations between the subjective and endocrine measures provide preliminary support for a role of these endogenous steroids in some of the subjective effects of alcohol.

In a recent work Ford et al. [357] hypothesized that inhibition of 5a-reduced neurosteroid biosynthesis would hinder the acquisition of ethanol intake, presumably by attenuating positive modulatory tone at GABA-A receptors. Thus, these investigators examined the effect of subchronic FIN administration on the acquisition of limited access ethanol preference drinking in male B6 mice. They found that FIN dose-dependently blocked the acquisition of drinking and prevented the development of ethanol preference, thereby suggesting that the GABAergic neurosteroids may be important in the establishment of stable drinking patterns. FIN-treated mice continued to exhibit attenuated ethanol consumption after 2 weeks post-treatment, despite a full recovery in brain ALLO levels. This study suggests that FIN-mediated modulation of the GABAergic system may be useful in curbing ethanol intake acquisition [357].

IS THERE A PLACE FOR NON-PHARMACOLOGICAL NEUROBIOLOGICAL APPROACHES?

Although beyond the goals of this review, we want to mention the recent and growing interest for some techniques, such as the transcranial magnetic stimulation (TMS). In fact, “boosting” the DA system should ameliorate compulsive drug/alcohol use [358], and this is the principle how TMS might work alcohol consumption. Transcranial magnetic stimulation is the first non-invasive non-pharmacological therapeutic approach to mental/brain disorders [359]. As such TMS promises to be an innovative approach to alcohol and other addictions with a negligible range of systemic side-effects and limited contraindications. In particular, recent technological developments such as the H-coil [360] allow modulation of brain regions as deep as 6 cm below cortical surface [360] thereby making the NAc accessible to external modulation.

CONCLUSIONS

This review has presented and discussed a variety of studies that have investigated possible new targets in the treatment of AD. These studies demonstrate a growing scientific excitement and complexity aimed at finding new pharmacotherapies for AD. Alcohol dependence is now considered a medical disease, but unlike other medical problems (hypertension, diabetes, just to cite few), only a few medications are available. Thus, there is a crucial need to develop new more effective pharmacotherapies for the treatment of AD patients. Future research will aim to translate the present data into clinical settings. However, translating preclinical into clinical success has its own challenges as we note that in some instances there is discordance between animal and human studies. Additionally there is a need to identify specific typologies of alcoholics who may benefit from specific pharmacotherapies [361]. In summary, future research must seek to identify not only efficacious medications, but also - and more importantly - to delineate the best match between a particular pharmacotherapy and a specific alcoholic typology.

ABBREVIATIONS

\[\text{αCtxMII} = \text{α-conotoxin MII}\]
\[\text{18-MC} = \text{18-Methoxycoronaridine}\]
\[\text{ACD} = \text{Acetaldehyde}\]
\[\text{Ach} = \text{Nicotinic acetylcholine}\]
\[\text{ACTH} = \text{Adrenocorticotropic hormone}\]
\[\text{AD} = \text{Alcohol dependence}\]
\[\text{ADH} = \text{Alcohol dehydrogenase}\]
\[\text{ALDH-2} = \text{Aldehyde dehydrogenase \text{2}}\]
\[\text{ALLO} = \text{Allopregnanolone}\]
\[\text{AMPA} = \text{Methylisoxazole-4-propionic acid}\]
\[\text{ARC} = \text{Arcuate nucleus}\]
\[\text{AVP} = \text{Arginine vasopressin}\]
\[\text{BK or MaxiK} = \text{Large conductance}\]
\[\text{Ca}^{2+} = \text{Calcium}\]
\[\text{CAK} = \text{Calcium-activated-potassium}\]
\[\text{CB} = \text{Cannabinoid}\]
\[\text{CeA} = \text{Central nucleus of amygdala}\]
\[\text{CFH} = \text{Corticotropin-releasing hormone}\]
\[\text{CHRM2} = \text{Muscarinic acetylcholine M2 receptor}\]
\[\text{CNS} = \text{Central nervous system}\]
\[\text{COGA} = \text{Collaborative Study on the Genetics of Alcoholism}\]
\[\text{CPP} = \text{Conditioned place preference}\]
\[\text{CRF} = \text{Corticotropin-releasing factor}\]
\[\text{CRFR} = \text{Corticotropin-releasing factor receptor}\]
\[\text{CYP2E1} = \text{Cytochrome P4502E1}\]
\[\text{DA} = \text{Dopamine}\]
\[\text{DHβE} = \text{Dihydro-β-erythroidine}\]
\[\text{DR} = \text{Dorsal raphe}\]
\[\text{FDA} = \text{Food and Drug Administration}\]
FIN = Finasteride
GABA = γ-aminobutyric acid
GalR = Galanin receptor
GDNF = Glial cell line-derived neurotrophic factor
GH = Growth hormone
GHS-R = Growth hormone secretagogue receptor
GlyR = Glycine receptor
iGluR = Ionotropic glutamate receptor
i.p. = Intraperitoneally
i.c.v. = Intracerebroventricular
IK = Intermediate conductance
ITF = Inducible transcription factor
K+ = Potassium
KA = Kainic acid
LAH = Lateral hypothalamus
LC = Locus coeruleus
LDTg = Laterodorsal tegmental nucleus
LH = Luteinizing hormone
LHRH = Luteinizing hormone releasing hormone
LS = Lateral septum
mACHR = Muscarinic acetylcholine receptor
MC = Melanocortins
MeA = Medial nucleus of amygdala
mGlur = Metabotropic glutamate receptor
MLA = Methyllycaconitine
MPEP = 2-Methyl-6-(phenylethynyl)-pyridine
MPZP = (N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethylpyrazolo[1,5a]pyrimidin-7-amine)
MSH = Melanocyte stimulating hormone
MTIP = 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-ethynyl]-2,6-dimethyl-imidazo[1,2-b]pyridazine
N/OFQ = Nociceptin/Orphanin FQ
Nac = Nucleus accumbens
nAChR = Nicotinic acetylcholine receptor
NAM = Negative allosteric modulator
NE = Norepinephrine
NE = Norepinephrine system
NK1R = Neurokinin 1 receptor
NK3R = Tachykinin receptor 3
NMDA = N-methyl-D-aspartic acid
NOP = Nociceptin/orphanin peptide
NP078585 = 1-(6,6-bis(4-fluorophenyl)hexyl)-4-(3,4,5-trimethoxybenzyl) piperazine
npEW = Non-preganglionic Edinger-Westphal
NPS = Neuropeptide S
NPY = Neuropeptide Y
ORL-1 = Opioid receptor-like 1
pIIu = Periculomotor urocortin
POMC = Proopiomelanocortin
PTSD = Posttraumatic Stress Disorder
PVN = Paraventricular nucleus
REM = Rapid eye movement
Ret = Transfection receptor
SigR = Sigma-receptor
SK = Small conductance
SNc = Substantia nigra pars compacta
SP = Substance P
STAI = Spielberg Trait Anxiety Inventory Test
TH = Tyrosine hydroxylase
THDOC = Tetrahydrodeoxycorticosterone
TMS = Transcranial magnetic stimulation
Ucn = Urocortin
US = United States
VGCC = Voltage-gated calcium channels
VTA = Ventral tegmental area

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