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Review

Translating the neuroscience of alcoholism into clinical treatments: From blocking the buzz to curing the blues

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ABSTRACT

Understanding the pathophysiology of addictive disorders is critical for development of new treatments. A major focus of addiction research has for a long time been on systems that mediate acute positively reinforcing effects of addictive drugs, most prominently the mesolimbic dopaminergic (DA) system and its connections. This research line has been successful in shedding light on the physiology of both natural and drug reward, but has not led to therapeutic breakthroughs. The role of classical reward systems is perhaps least clear in alcohol addiction. Here, recent work is summarized that points to some clinically important conclusions. First, important pharmacogenetic differences exist with regard to positively reinforcing effects of alcohol and the ability of this drug to activate classical reward pathways. This offers an opportunity for personalized treatment approaches in alcoholism. Second, brain stress and fear systems become pathologically activated in later stages of alcoholism and their activation is a major influence in escalation of alcohol intake, sensitization of stress responses, and susceptibility to relapse. These findings offer a new category of treatment mechanisms. Corticotropin-releasing hormone (CRH) signaling through CRH1 receptors is a major candidate target in this category, but recent data indicate that antagonists for substance P (SP) neurokinin 1 (NK1) receptors may have a similar potential.

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1. Introduction: alcohol is different

Although increasingly refined, current theories of addiction largely continue to build on two major sets of discoveries. The first of these are the findings identifying the mesolimbic dopaminergic (DA) pathway as a critical substrate for motivation to engage in exploration of the environment and pursuit of goal oriented behavior. The second set consists of observations that most, if not all, addictive drugs interact with this DA circuitry.

Combining these insights, a succession of theories have focused on drug—DA interactions to account for the narrowing of the behavioral repertoire and its skewing toward drug seeking characteristics of clinical addiction. According to early versions of these DA-centric views, addictive drugs activate DA signaling with a supraphysiological amplitude and are, therefore, able to successfully compete with natural rewards and also lead to

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conditioned reinforcement from drug-associated cues (Stewart et al., 1984; Wise and Bozarth, 1985; Koob et al., 1987; Di Chiara and Imperato, 1988). In increasingly sophisticated iterations that have followed, the mesolimbic DA system is considered critical through its role to signal the expectation of reward (Schultz, 1998), its mediation of pathological Pavlovian learning thought to be unique to addictive drugs (Di Chiara, 1999), or sensitization of incentive salience for drug-associated stimuli even while actual drug reward undergoes tolerance (Robinson and Berridge, 2003). Additional views hold that pre-existing differences in dopamine receptor endowment determine vulnerability to drug reinforcement (Volkow and Li, 2004).

This succession in part reflects an increasing realization that acute positively reinforcing effects of addictive drugs may play an important role in early stages of addiction, but that addiction is a chronic relapsing disorder, ultimately maintained by pathology of brain function that arises over time, and only in a minority of subjects that initially engage in drug use (McLellan et al., 2000). Perhaps at the extreme of this spectrum is the recent view that drug seeking and taking ultimately becomes habitual, and essentially disengaged from normal motivational control (Belin et al., 2009). More broadly, current views on the significance of DA transmission in development of addiction tend to focus on the role of dopamine (DA) in associative learning. In this view, DA release could serve as a direct indicator of reward only in early phases of addiction, while in the longer term, the most important role of DA is to mediate the associative learning that increases the salience of environmental cues present during drug taking.

The discoveries briefly outlined above reflect a major success story of modern neuroscience, and have provided critical insights into the physiology of brain systems that mediate natural reward and learning. They have, however, not translated into major advances in clinical treatment of addictive disorders. Stabilization and maintenance of heroin addiction with the long acting opiate agonist methadone, and the conceptually related partial agonist buprenorphine, remain unparalleled as major success stories of clinical addiction medicine (Amato et al., 2005). Clinicians that continue to be faced with unmet medical needs in the area of addictive disorders are therefore forced to ask whether looking beyond systems that mediate acute drug reward might be necessary to understand the pathophysiology of addictive disorders and to identify clinically useful treatment targets.

This question is particularly relevant in the area of alcohol addiction. Central stimulants and cocaine have well established, potent and direct effects on DA transmission, and it is therefore not unreasonable to hypothesize that interactions with DA systems may be critical for their addictive properties (Wise and Bozarth, 1985). Opioids also potently modulate DA transmission, although this action is indirect, and it is clear that activation of opioid receptors can be reinforcing both in DA-dependent and DA-independent ways (Amalric and Koob, 1985; Johnson and North, 1992; Spanagel et al., 1992). In contrast, alcohol modulates a wider range of neurochemical systems than perhaps any other addictive drug (Spanagel, 2009). Any of these systems could mediate neuroadaptations leading to an alcohol addicted state, and evidence for a unique role of DA transmission in alcohol addiction is not present.

In the following, research will be reviewed from which some potentially clinically relevant insights are emerging. The key points of this synthesis are the following (Fig. 1):

- Acute positively reinforcing properties of alcohol are predominantly mediated through indirect effects on mesolimbic DA, downstream of endogenous opioid peptides release in response to alcohol.
- These positively reinforcing alcohol actions are likely to be most relevant in early stages of the addictive process, particularly among individuals with genetic susceptibility.
- Under these conditions, alcohol associated cues are predictive of positively reinforcing alcohol effects, leading to what can be called "reward craving".

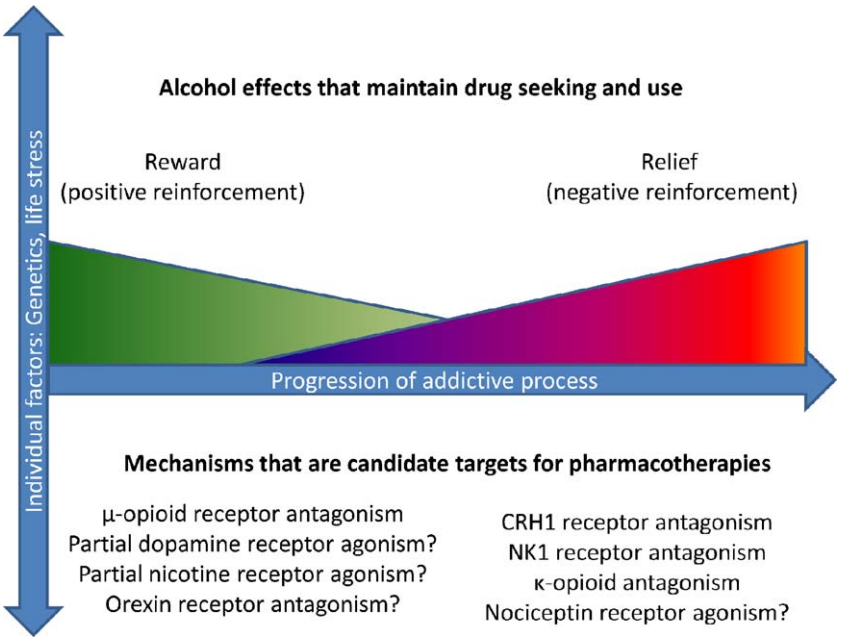

Fig. 1. As the addictive process evolves, the motivational mechanisms that drive alcohol seeking and relapse to excessive alcohol use shift. Initially, alcohol use is largely driven by pleasurable, positively reinforcing alcohol effects. As the alcohol addicted brain develops progressive neuroadaptations, there is a shift toward mechanisms that mediate relief from a negative emotional state that is experienced in the absence of drug. The relative weight of the respective category of motivational mechanisms will depend both on how far into this process an individual is, but also on individual susceptibility factors such as genetic makeup and stress exposure. Depending on these factors, personalized medicine approaches to alcoholism treatment will have to target different mechanisms. While the focus of the present review is on treatments targeting μ-opioid, CRH1 and NK1 receptors, other mechanisms within the respective category are also listed in the figure.

- With prolonged excessive use of alcohol, brain stress systems become progressively engaged and sensitized, leading to further escalation of alcohol use and increased vulnerability to relapse, at this stage driven by negative reinforcement and craving for negatively reinforcing alcohol actions, or "relief craving".
- This conceptualization points to the need for personalized medicine approaches to alcoholism treatment, in each case tailored to unique patient factors, including genetics and history.

2. Getting the buzz from alcohol: an opioid–DA cascade

In trying to understand to what extent alcohol interactions with classical reward pathways are a critical substrate for reinforcement of alcohol seeking and intake, several observations need to be made. Already in 1973, a seminal but often overlooked human study suggested a key role for DA in pleasurable and stimulating effects of alcohol in healthy, non-dependent volunteers (Ahlenius et al., 1973). Numerous microdialysis studies in rodents subsequently demonstrated an ability of alcohol to release DA in the Nc. Accumbens, although the magnitude of this release is markedly lower than that found in response to, e.g. central stimulants (Imperato and Di Chiara, 1986; Di Chiara and Imperato, 1988). Of interest given human data discussed below, alcohol-induced DA release was found to be much greater in genetically selected alcohol preferring rats than in non-selected Wistar rats (Weiss et al., 1993). In humans, a pharmacokinetically controlled, intravenous (i.v.) infusion of alcohol has been shown to activate the ventral striatum of non-dependent social drinkers, as measured by functional magnetic resonance tomography (fMRI) (Gilman et al., 2008). Consistent with this observation, positron emission tomography (PET) using displacement of the D2 receptor ligand ^{11}C-raclopride has also directly shown a modest magnitude of DA release in response to an oral alcohol challenge in social drinkers (Boileau et al., 2003), although this was not replicated by others (Yoder et al., 2007).

It thus appears reasonably well established that alcohol is in fact capable of activating classical dopaminergic reward pathways, albeit less potently than prototypical addictive drugs. This observation does, however, not directly address two critical questions. It is unclear to what extent activation of DA transmission by alcohol is causally related to alcohol seeking and use. Furthermore, it is not well established whether such an activation persists into the addicted state. Two sets of observations have long suggested a partial contribution at best, and one that may not to a large extent persist into late stages of addiction. First, it has long been known that near-complete lesions of DA input to the Nc Accumbens in rats leave alcohol intake as well as operant responding for alcohol essentially unaffected (Kiianmaa et al., 1979; Rassnick et al., 1993b). Furthermore, preliminary data suggest that activation of ventral striatum by alcohol, as measured by fMRI, is much less pronounced in alcohol-dependent subjects compared to non-dependent social drinkers (Gilman et al., 2009).

To the extent alcohol does activate classical brain reward pathways, it appears to a major extent to do so indirectly, through a cascade that involves release of endogenous opioid peptides in the ventral tegmental area (VTA), resulting in disinhibition of DA neurons in this region (Di Chiara et al., 1996; Tanda and Di Chiara, 1998; Jarjour et al., 2009). This offers an opportunity to assess the functional importance of the alcohol–opioid–DA cascade without a confound from non-specific motor effects that are typically associated with direct blockade of DA transmission. The prediction to be tested is that if activation of classical brain reward circuitry indeed does contribute to excessive alcohol use, then its blockade should hold some therapeutic potential. In agreement with this prediction, initial observations in non-human primates (Altshuler et al., 1980) were followed by the discovery of the opioid receptor antagonist naltrexone as an alcoholism treatment (O'Malley et al., 1992; Volpicelli et al., 1992). Two decades later, despite some negative studies, meta-analyses of more than 30 randomized controlled trials support the efficacy of this treatment (Bouza et al., 2004).

The same meta-analysis, however, supports only a modest effect size of naltrexone. One possible conclusion from this observation is that classical reward system activation by alcohol only plays a minor role in excessive alcohol use and alcoholism. If this is correct, then there is no strong rationale for clinical use of treatments that target alcohol reward. Unfortunately, and as we will show largely incorrectly, this is the conclusion that for the most part has been drawn by clinicians. Based on this conclusion, clinical use of naltrexone has not become widespread (Mark et al., 2003). The alternative interpretation is that the limited overall effect size of naltrexone reflects heterogeneity of response among patients. Clinical experience and accumulating research data in fact point to the latter scenario. Specifically, secondary analyses of clinical trials suggest that family history of alcoholism predicts clinical naltrexone response (Rubio et al., 2005). Direct support for this notion is also found under laboratory conditions, both with regard to subjective alcohol effects (King et al., 1997) and alcohol self-administration (Krishnan-Sarin et al., 2007). Although a role of family history clearly could reflect either genetic or environment factors or both, emerging evidence strongly suggests a major role of pharmacogenetic factors.

Animal data indicate that, among the three cloned opioid receptors, the μ-subtype is critical for alcohol reinforcement. Genetic disruption of the μ-opioid receptor gene (*OPRM1*) results in a loss of alcohol self-administration in mice, while pharmacological blockade of this subtype decreases ethanol drinking both in non-selected Wistar rats and in rat lines bred for high alcohol preference [reviewed in (Heilig and Egli, 2006)]. Genetic variation at the *OPRM1* locus is therefore an obvious candidate as a potential pharmacogenetic determinant of both alcohol and naltrexone responses. An A118G *OPRM1* SNP discovered over a decade ago encodes an amino acid (a.a.) substitution at a glycosylation site located in the N-terminal extracellular arm of the receptor (Bond et al., 1998), and is therefore potentially functional. Although we have found replicable associations between the *OPRM1* 118G polymorphism and addictive disorders in Swedish cohorts with little if any ethnic admixture (Bart et al., 2004, 2005), this variant in general remains controversial as a genetic susceptibility factor for alcoholism (Arias et al., 2006). Leaving this issue aside, *OPRM1* 118G is more consistently found to modulate responses to alcohol and to μ-opioid receptor blockade (Wand et al., 2002; Ray and Hutchison, 2007; Kakko et al., 2008). Some secondary analyses of published clinical trials also suggest that *OPRM1* 118G carriers are particularly responsive to naltrexone (Oslin et al., 2003; Anton et al., 2008), but results have not been consistent (Gelernter et al., 2007).

Clinical evaluation of pharmacogenetic factors poses numerous challenges unless studies are specifically designed to detect them. Most fundamentally, unless subjects are *a priori* recruited based on genotype, there is always a bias against detecting effects confined to carriers of a minor allele. Studies in non-human primates have therefore offered a valuable complement to address this set of questions. An *OPRM1* SNP that is functionally equivalent to the human A118G polymorphism (C77G) has been identified in rhesus macaques (Miller et al., 2004). Using this model, we found increased psychomotor stimulation in response to alcohol, increased alcohol preference, and increased frequency of alcohol consumption at a level leading to intoxication in carriers of the rhesus (rh) *OPRM1* 77G variant (Barr et al., 2007). These findings suggested that activation of classical brain reward systems in response to alcohol primarily or perhaps even exclusively occurs in carriers of the rhesus 77G variant.

A testable hypothesis prompted by these findings was that 77G carriers should be preferentially sensitive to suppression of alcohol preference by naltrexone. We used a short-term treatment model and social drinking in non-dependent rhesus macaques to evaluate this hypothesis. In agreement with our prediction, naltrexone only suppressed alcohol preference in carriers of the rhesus 77G variant (Barr et al., 2009a). Both the rhesus and the human data may have their own limitations, but they are highly complementary. Together, the picture that emerges is consistent with that suggested by the human secondary analyses that support a role of 118G as a predictor of treatment efficacy (Oslin et al., 2003; Anton et al., 2008).

The non-human primate and human data are also complementary in another aspect, in that they allow isolating the influence of C77G (in rhesus) and A118G (in humans) from that of other functional polymorphisms with which the respective variants might be in linkage disequilibrium (LD) in the two species. For instance, one human study found that other polymorphisms within the same haplotype block, but not A118G, were associated with diagnoses of substance dependence (Zhang et al., 2006). In contrast, a haplotype-based re-analysis of the COMBINE study found naltrexone response to be specifically attributable to 118G (Oroszi et al., 2009). Furthermore, in humans, alternative isoforms of the μ-opioid receptor are encoded by transcripts that originate from different initiation sites, and genotype may therefore serve as a proxy for isoform identity (Shabalina et al., 2009). Combined, however, the human and rhesus findings strongly suggest that the *rhOPRM1* C77G and the *hOPRM1* A118G SNPs, respectively, are functional with regard to alcohol as well as naltrexone response in the respective species.

Interestingly, our rhesus study in fact found opposite directionality of the naltrexone effect in 77G carriers and subjects homozygous for the major 77C allele. While alcohol preference was markedly suppressed in 77G carriers, there was a trend for increased preference in 77C homozygous individuals. This pattern parallels a human study that examined family history of alcoholism as a moderator of naltrexone response under laboratory conditions, and found suppression of self-administration in family history positive subjects, but significantly *increased* self-administration following naltrexone treatment in family history negative participants (Krishnan-Sarin et al., 2007). These data are consistent with a direct assessment of alcohol-induced DA activity. In a human PET study using ^{11}C-raclopride displacement, we recently studied the release of DA in response to a pharmacokinetically controlled alcohol challenge in non-dependent, social drinkers, and evaluated whether it varies as a function of the human *OPRM1* A118G genotype. Throughout the striatum, displacement of the radioligand was only detected in 118G carriers, while the same measure in fact suggested *reduced* DA release in subjects homozygous for the major 118A allele following alcohol challenge (Ramchandani et al., 2009).

Finally, classical studies in offspring of alcoholics [recently reviewed in (Schuckit, 2009)] have established that a low innate sedative—ataxic response to alcohol is a key heritable susceptibility factor for alcohol use disorders. The relationship between this trait and the pharmacogenetic variation in alcohol responses reviewed above is presently unclear. The simplest possibility is that low response to sedative—ataxic alcohol actions and high response to alcohol reward reflect two independent heritable factors. Alternatively, *OPRM1* variation could contribute to both these intermediate phenotypes. In support of this possibility, family history positive subjects also have increased cortisol responses to an naltrexone challenge (Wand et al., 1998), and the same intermediate phenotype is found in carriers of the *OPRM1* 118G allele (Wand et al., 2002). Finally, separate genetic factors could confer low sedative—ataxic and high rewarding responses to alcohol, but an important functional link may arise between these traits. Genotype at the *OPRM1* locus determines the dose–response relationship for alcohol reward, such that 118G carriers show a left-shift of this function. In contrast, genetic factors that modulate the dose–response relationship for sedative alcohol actions determine at what dose level these effects will impose a limit on alcohol seeking and consumption. These two factors will therefore interact to determine whether an individual can consume alcohol to a level that will produce sufficiently potent rewarding actions.

In summary, a highly consistent picture seems to be emerging from these studies with regard to interactions of alcohol with classical brain reward circuitry:

- First, such interactions are for the most part indirect, and rely on alcohol-induced release of endogenous opioids and their actions at opioid receptors for activation of mesolimbic DA.
- Second, acutely reinforcing actions of alcohol through this cascade are more pronounced in early than in late stages of the addictive process.
- Third, these actions are particularly relevant, and perhaps remain so into later stages of addiction, in individuals with genetic susceptibility factors, among which genetic variation at the OPRM1 locus appears to be key.
- Fourth, in susceptible individuals, blockade of alcohol-induced reward seems to be a mechanism that can be targeted therapeutically with considerable benefits. In contrast, this strategy seems to be of limited if any utility in subjects without the susceptibility factors.

3. Curing the blues: extrahypothalamic corticotropin-releasing hormone (CRH)

What, then, maintains excessive alcohol use under conditions when acute rewarding alcohol actions are less important? Clearly, acute tension-reducing or anxiolytic alcohol effects are likely to contribute to social alcohol use. Alcohol actions on GABA-ergic and glutamatergic transmission are major mediators of these acute negatively reinforcing alcohol effects. These mechanisms have recently been reviewed (Spanagel, 2009) and are beyond the scope of the present paper. Because only a minority of people who establish regular alcohol use habits develops alcoholism (Hasin et al., 2007), other processes must presumably kick in as this disease develops. Indeed, a central theme that has emerged from recent research is that progression from social alcohol consumption into alcoholism is characterized by extensive long-term changes in brain function, or neuroadaptations, that induce and maintain the addicted state.

Studies of long-term neuroadaptations in alcohol addiction have long been limited by methodological difficulties. In contrast to, e.g. cocaine or heroin, sufficient levels of voluntary alcohol consumption to induce dependence cannot be easily achieved in most species of experimental animals. For most species and most individuals, oral alcohol is simply not a very potent acute reinforcer (Egli, 2005). A practical solution to this dilemma has been offered by the use of alcohol vapor inhalation, a model first established in the seventies (Goldstein and Pal, 1971). Vapor inhalation allows precise control of brain alcohol exposure and makes it possible to emulate a level, pattern and duration of exposure that shares key characteristics with what occurs in clinical alcoholism. Using this approach, it has been shown that prolonged brain alcohol exposure at intoxicating levels leads to persistent behavioral consequences that seem to be relevant for alcoholism (Roberts et al., 2000; Rimondini et al., 2002). A prolonged duration (Rimondini et al., 2003) and an intermittent pattern of exposure (Rimondini et al., 2002; O'Dell et al., 2004), two

features that mimic the exposure profile in clinical alcoholism, appear critical for induction of the behavioral changes. Other methods that lead to repeated cycles of intoxication and withdrawal exist, such as, e.g. through forced liquid diet. Although perhaps less potent and less easy to control, these can induce a similar set of behavioral consequences [for review, see (Breese et al., 2005a)].

Prolonged brain alcohol exposure in experimental animals results in two key behavioral consequences:

- Escalation, i.e. progressive increase of subsequent voluntary alcohol intake, measured both using simple two-bottle free-choice drinking (Rimondini et al., 2002; Lopez and Becker, 2005; Lopez et al., 2008; Griffin et al., 2009) and operant responding for alcohol (Roberts et al., 2000).
- Sensitization of behavioral stress responses (Overstreet et al., 2002; Valdez et al., 2002, 2003, 2004; Breese et al., 2005b; Sommer et al., 2008).

Since a landmark paper (Ahmed and Koob, 1998), escalation has become virtually synonymous with increase in self-administration that occurs with extended access to drug. It is therefore important to notice that, in what may be another important difference between alcohol and other addictive drugs, experimenter-imposed brain alcohol exposure is sufficient to induce a similar progressive increase in voluntary drug intake. Following a prolonged history of dependence in this model, both escalation and behavioral sensitization to stress emerge during withdrawal, but persist long after withdrawal symptoms have resolved, and may in fact be very long lasting. The term "post-dependent" has been introduced to reflect the sum of neuroadaptations that are induced as an individual becomes dependent on alcohol, and that remain for extended periods of time thereafter. These neuroadaptations can be maintained by continued brain alcohol exposure, but one of their key characteristics is that they remain even in its absence. Although not studied in detail until now, the extent and duration of post-dependent neuroadaptations show considerable individual variability. It can be hypothesized that, based on genetic susceptibility and other factors, the neuroadapted, post-dependent state remains indefinitely in some individuals ("once an alcoholic, always an alcoholic"), while in others it remits.

Among the two behavioral characteristics of the post-dependent state, escalation of intake obviously mirrors a core characteristic of clinical alcoholism. Persistent sensitization to stress has now consistently been shown using the elevated plus-maze (Valdez et al., 2003), social interaction (Overstreet et al., 2002) and fear-suppressed (conflict) responding in rats (Sommer et al., 2008). These observations are interesting given that anxiety symptoms seem to be present regardless of alcoholism subtype (Ducci et al., 2007), but are highly contentious in clinical alcoholism research [see e.g. (Schuckit and Hesselbrock, 1994)]. The dynamic nature of dependence-induced sensitization to stress is probably key to resolving this issue. The following set of observations provides perhaps the clearest demonstration of this pattern (Valdez et al., 2003). Seven weeks after completion of alcohol exposure, a history of dependence did not seem to result in any anxiogenic effect on the elevated plus-maze under unchallenged conditions. This is in agreement with clinical observations, in which established clinical ratings of anxiety in most patients decline over 3–6 weeks to clinically insignificant levels (Schuckit and Hesselbrock, 1994). A very different picture emerged, however, when plus-maze testing was preceded by a restraint stress. Importantly, the magnitude of the stressor was chosen so that it did not result in an anxiogenic effect in animals without a history of alcohol exposure. In animals with a prolonged history of alcohol dependence, however, a potent anxiogenic effect was observed in this case. In a conflict model, where the anxiety testing itself is carried out under stressful conditions, the sensitized response of animals with a history of dependence is observed without additional manipulations (Sommer et al., 2008).

Some human translation of these findings is already available. Detoxified alcoholics have up-regulated brain responses to negative affective stimuli from the International Affective Picture System [IAPS; (Lang et al., 1995)], as measured by fMRI (Gilman and Hommer, 2008). Interestingly, a key component of the network where sensitized activation is seen is the insula, a brain region that encodes negative interoceptive states, and whose anterior part has a high degree of reciprocal connectivity with the amygdala complex (Naqvi and Bechara, 2009). Insula activation correlates with subjective measures of craving (Brody et al., 2002), and loss of this structure caused a remarkable disruption of cigarette smoking (Naqvi et al., 2007). The sensitized brain responses to negative IAPS pictures were observed a minimum of 3 weeks into abstinence, a time point at which conventional anxiety ratings are typically back to clinically insignificant levels. Thus, taking together the animal and the human observations, the post-dependent state may not necessarily be characterized by an increased anxiety, but rather by up-regulated reactivity to stressors. Given the critical role of stressors to trigger relapse (Brownell et al., 1986; Shaham et al., 2003), this is likely of importance for relapse vulnerability.

A history of dependence also appears to link sensitization of stress responses to escalation of voluntary alcohol intake. A common perception holds that stress generally increases alcohol intake, but this phenomenon is in fact not typically observed in non-dependent animals [see e.g. (Vengeliene et al., 2003)]. The picture, however, becomes very different following a history of dependence. As indicated above, even under non-stressful conditions, these animals start out with a higher level of voluntary alcohol consumption. Following exposure to stress, they escalate their intake further, and maintain it at this higher level even after the stress exposure has been terminated (Sommer et al., 2008). It would therefore appear from these observations that long-term neuroadaptations in alcohol dependence not only lead to escalation of alcohol intake and sensitization of stress responses, but also create a connection between these two behaviors.

Recruitment of extrahypothalamic corticotropin-releasing hormone (CRH) transmission is a key neuroadaptive mechanism underlying the behavioral traits described above. CRH is best known as the hypothalamic release hormone for ACTH (Vale et al., 1981), but extensive networks of CRH expressing neurons are also present in extrahypothalamic structures, including the central nucleus of the amygdala (CeA) and bed nucleus of stria terminalis (BNST), two components of the extended amygdale that are critical for stress responses and emotionality (Swanson et al., 1983). Behavioral stress responses are largely mediated by extrahypothamic CRH_1 receptors, primarily in the amygdala and BNST. Effects of CRH_2 activation are more variable and region dependent, but are commonly opposite to those of CRH_1 (Makino et al., 2002; Muller and Wurst, 2004).

A critical feature of CRH signaling in behavioral stress responses may help understand the dynamic, activity dependent nature of sensitized stress responses in later stages of alcoholism. Neuropeptide systems are commonly released only at high neuronal firing frequencies, making them "alarm systems" that are not engaged under physiological or near-physiological conditions (Hokfelt et al., 1984). In agreement with this principle, extrahypothalamic CRH signaling seems to be largely quiescent unless activated by exposure to uncontrollable stress (Griebel et al., 2002; Gully et al., 2002).

Evidence has long been available to show that CRH activity within the amygdala and/or BNST drives acute alcohol withdrawal

anxiety (Merlo et al., 1995; Olive et al., 2002; Baldwin et al., 1991; Rassnick et al., 1993a; Gehlert et al., 2007). Of greater importance for the chronically addicted state, recent advances have shown that the persistent sensitization of behavioral stress responses following a history of dependence, described above, is also driven by CRH activity (Valdez et al., 2003; Funk et al., 2006a, 2007; Sommer et al., 2008). Similar results have been obtained using other means to induce alcohol dependence and the associated long-term neuroadaptations (Overstreet et al., 2002, 2004; Knapp et al., 2004; Breese et al., 2005b).

Similar to the sensitized stress responses, escalated self-administration or intake of alcohol following a history of dependence are also driven by up-regulated activity of extrahypothalamic CRH. Post-dependent animals tested two hours into withdrawal exhibit markedly elevated rates of self-administration, and these are brought down to non-dependent levels by systemic treatment with a whole range of CRH_1 selective antagonists. Showing the different nature of escalated vs. baseline alcohol self-administration, none of the antagonists affected self-administration in non-dependent animals (Funk et al., 2007). In a follow-up study, the non-selective peptide CRH antagonist D-Phe $CRH_{12–41}$ microinjected into the CeA blocked excessive post-dependent self-administration rates, while microinjections into BNST or the Nc. Accumbens shell were ineffective. Furthermore, CeA injections of D-Phe CRH_{12-41} in animals without a history of dependence were also ineffective, once again demonstrating that the CRH system, presumably within the amygdala, is recruited to drive excessive alcohol self-administration in the post-dependent state (Funk et al., 2006a).

Suppression of escalated alcohol self-administration by CRH antagonism as outlined above was observed during acute withdrawal, but escalated alcohol self-administration has also been found long after forced alcohol exposure, and is equally sensitive to CRH or CRH_1 antagonism (Rimondini et al., 2002; Valdez et al., 2002; Gehlert et al., 2007). Intracerebroventricular administration of a CRH antagonist blocked escalated alcohol intake both during acute withdrawal and protracted abstinence, but did not affect basal alcohol intake in animals without a history of dependence (Valdez et al., 2002). Similarly, dependence induction using gastric gavage, followed by cycles of self-administration and imposed deprivation periods, also resulted in excessive self-administration. After several weeks, the novel selective non-peptide CRH_1 antagonist MTIP suppressed alcohol self-administration in post-dependent animals to non-dependent levels, while the same doses of MTIP were inactive in animals without a history of dependence (Gehlert et al., 2007). In summary, a pathological engagement of extrahypothalamic CRH activity drives escalated alcohol intake in animals with a history of dependence, both during withdrawal and long after withdrawal has subsided.

As indicated above, stress is a major trigger for relapse in alcoholics, and reinstates previously extinguished alcohol seeking in experimental animals (Brownell et al., 1986; Shaham et al., 2003). Both non-selective and CRH_1 selective CRH antagonists block stress-induced reinstatement, but do not influence reinstatement triggered by alcohol associated stimuli, which in contrast is blocked by naltrexone. The ability of CRH blockade to suppress stress-induced relapse-like behavior is mediated through extrahypothalamic CRH systems (Le et al., 2000; Liu and Weiss, 2002). Post-dependent animals display a markedly increased sensitivity to blockade of stress-induced reinstatement by CRH antagonism (Gehlert et al., 2007). The selective CRH_1 antagonist MTIP entirely blocked this behavior at 10 mg/kg, a dose at which no effect was seen in animals without a history of dependence. Taken together, these data show that CRH_1 receptors mediate stress-induced reinstatement, and that a recruitment of the CRH system in the post-dependent state renders animals preferentially sensitive to blockade of relapse-like behavior by CRH_1 antagonism.

The α_2-adrenergic antagonist yohimbine, a pharmacological stressor that can substitute for foot-shock to reinstate alcohol seeking (Le et al., 2005), has recently been shown to up-regulate CRH expression in CeA (Funk et al., 2006b). It is, however, unknown whether CRH within CeA mediates stress-induced reinstatement, while CRH antagonist microinjections into the median raphe blocked relapse-like behavior in this model (Le et al., 2002). This suggests that multiple CRH pathways might be involved and act in concert to mediate different alcohol-related behaviors.

Thus, recruitment of CRH signaling within the extended amygdala is a major factor behind increased stress sensitivity, excessive self-administration and relapse in the post-dependent state. The mechanisms through which this occurs are beginning to emerge. During acute alcohol withdrawal, release of CRH is increased in the amygdala (Merlo et al., 1995). Presumably as a reflection of this, decreased tissue levels of CRH were seen within this structure in early withdrawal (Zorrilla et al., 2001; Funk et al., 2006a). Six weeks after last alcohol exposure, however, amygdala CRH had not only recovered, but also increased to supranormal levels (Zorrilla et al., 2001). Elevated tissue content of CRH peptide in the amygdala could either reflect increased synthesis, or decreased utilization. Our finding of increased CRH transcript levels in the CeA during the post-dependent state supports increased synthesis in CeA in this condition (Sommer et al., 2008).

A major contribution to up-regulated CRH signaling, however, comes from an up-regulation of CRH_1 receptor expression and binding within the amygdala. This is consistent with the left-shifted dose–response curve for CRH_1 antagonists observed in animals with a history of dependence. Perhaps the best demonstration that CRH_1 up-regulation produces the characteristics of the post-dependent phenotype was obtained in the genetically selected, alcohol preferring Marchigian–Sardinian Preferring (msP) rat (Ciccocioppo et al., 2006). These animals essentially represent a behavioral phenocopy of post-dependent rats, with which they share increased stress reactivity, excessive self-administration of alcohol, and increased propensity for relapse-like behavior. A screen for differential gene expression in the msP rat showed a marked up-regulation of the transcript encoding the CRH_1 receptor within the amygdala complex. This was linked to a *Crhr1* promoter variant unique to msP rats. In msP rats, the selective CRH_1 antagonist, antalarmin, reduced alcohol self-administration to levels found in genetically heterogeneous animals without a history of dependence. Antalarmin also blocked stress-induced reinstatement of alcohol seeking in msP rats at doses that did not affect non-selected rats without a history of dependence (Hansson et al., 2006). This is a further parallel to the post-dependent phenotype. Interestingly, when msP animals were given *ad lib* access to alcohol, the ensuing consumption was sufficient to down-regulate the receptor transcript to normal levels (Hansson et al., 2007). Genetic variation at the *Crhr1* locus as a susceptibility factor for excessive alcohol drinking might have parallels in primates, including rhesus macaques (Barr et al., 2009b) and humans, where a similar association was recently reported (Treutlein et al., 2006).

Following up on the msP findings, a similar up-regulation of CRH_1 expression was found in genetically non-selected, post-dependent rats (Sommer et al., 2008). This up-regulation persisted long after ethanol exposure, reflecting a long-term neuroadaptation rather than acute withdrawal. Similar to the msP findings, receptor up-regulation was most pronounced in the basolateral (BLA) and medial amygdala (MeA), and only to a lesser extent found in CeA (unpublished data). The relative contribution of specific amygdalar subnuclei to the post-dependent state remains to be established. Microinjections of a CRH antagonist in CeA blocked post-dependent excessive self-administration, but the BLA

or MeA were not tested in that study (Funk et al., 2006a). Also, amygdalar nuclei are interconnected, and receptors expressed in BLA or MeA neurons could be inserted into terminals in other amygdala regions.

In summary, neuroadaptations that occur after a prolonged history of alcohol dependence seem to persist long after brain alcohol exposure, and in some cases perhaps for the lifetime of the individual. From a clinical perspective, a persistent vulnerability even after extend period of sobriety has important implications for secondary prevention. Long-term neuroadaptations in alcoholism drive escalation of voluntary alcohol intake, behavioral sensitization to stress, and a concomitant sensitivity to stress-induced relapse. Up-regulation of CRH signaling within the amygdala complex appears to be a key mechanism behind these behavioral traits, and offers a promising target for new pharmacotherapies in alcoholism.

4. A (not so) new kid on the block: substance P and its NK1 receptor

Clinical translation of preclinical findings validating CRH1 receptors as a target for alcoholism treatment has been slow. This is largely related to issues of medicinal chemistry and is beyond the scope of the present paper. In the meantime, we have explored whether other stress systems might act in parallel with CRH to drive excessive alcohol intake. We have been particularly interested in mechanisms that have been in clinical development for other indications, and therefore might offer molecules for which target engagement and acceptable safety in humans has already been demonstrated.

Substance P (SP) and its neurokinin 1 receptor (NK1R) appeared to be of interest in this context. SP is an 11 amino acid peptide originally isolated from intestinal extracts in 1931 (Euler and Gaddum, 1931). For a long time, research on SP focused on nociceptive and inflammatory effects related to its role as a C-fiber sensory transmitter [for review, see (Payan, 1989)]. Hopes that blockers of SP transmission would become analgesic and anti-inflammatory treatments were, however, not fulfilled. Another category of possible indications was suggested by the fact that SP and its preferred NK1R are highly expressed in brain areas involved in stress responses, including the hypothalamus and the amygdala (Mantyh et al., 1984; Nakaya et al., 1994). Numerous observations also indicated a functional involvement of SP and NK1Rs in affective regulation. For instance, central injection of SP or related peptide agonists is anxiogenic in the elevated plus-maze (Teixeira et al., 1996) and causes conditioned place aversion (Elliott, 1988). Conversely, NK1R antagonism or genetic deletion of the receptor is anxiolytic- and antidepressant-like in animal models (Teixeira et al., 1996; File, 1997; Kramer et al., 1998; Papp et al., 2000; Rupniak et al., 2000; Santarelli et al., 2001; Rupniak et al., 2001; Ballard et al., 2001; Varty et al., 2002). Furthermore, activation of NK1R's resulting from release of endogenous SP has been linked to modulation of stress responses (Ebner et al., 2004, 2008a,b; Ebner and Singewald, 2007).

Based on these observations, NK1R antagonists have also been evaluated as possible therapeutics for affective disorders. However, despite initial findings in support of antidepressant and anxiolytic activity of NK1 antagonists in humans (Kramer et al., 1998; Kramer et al., 2004; Furmark et al., 2005), clinical efficacy for these indications has not been robustly replicated, and development has largely been discontinued. It is possible that NK1R signaling, while important in some depressed subjects, may not be sufficiently uniformly involved in depression to produce a robust efficacy signal in heterogeneous patient populations. A more consistent activation of this system may be present in alcohol addiction, similar to the CRH findings reviewed above, and might render NK1R antagonism a more effective target mechanism for this indication. In addition, it has been reported that deletion of the *Tacr1* gene that encodes the NK1R blocks opiate reward (Murtra et al., 2000; Ripley et al., 2002; Gadd et al., 2003). Because endogenous opioids in part mediate alcohol reward, modulation of opioid mechanism by NK1R's could represent an additional mechanism through which NK1R antagonists contribute to altered alcohol reward.

In exploring these possibilities, we first established that genetic deletion of NK1R suppressed alcohol intake in a simple two-bottle free-choice drinking model. A genetic inactivation strategy was initially chosen because of concern that available NK1R antagonists may have limited activity in rats and mice (Holmes et al., 2003). NK1R null mutant mice (De Felipe et al., 1998) were used after back-crossing into a C57BL/6 background for 10 generations, so that a sufficient level of voluntary alcohol consumption would be present in control animals to allow detection of suppression by the receptor gene deletion (Crabbe and Phillips, 2004). Wild-type (wt) control mice (NK1R+/+) ultimately consumed in excess of 10 g alcohol/kg/day at the end of a procedure in which alcohol concentration was gradually increased to 15% over 60 days. Alcohol consumption by NK1R−/− mice was markedly lower than that by wt controls. The difference was most prominent at higher alcohol concentrations, at which consumption motivated by pharmacological alcohol effects dominates over intake for non-pharmacological effects such as taste or calories (Crabbe and Phillips, 2004). Relative preference for alcohol was also markedly reduced, while water intake was unaffected by genotype (George et al., 2008).

Despite the concerns about cross-species activity mentioned above, we then found that in WT C57BL/6 mice, the NK1R antagonist L-703,606 suppresses alcohol intake in a manner that mimics the effects of genetically inactivating the NK1R. The antagonist was inactive in NK1R KO's, demonstrating that its effects to suppress alcohol drinking reflect activity at the target rather than off-target actions. Using a model recently developed by others (Melendez et al., 2006), we also found that escalation of alcohol intake after repeated cycles of deprivation was robustly detected in WT mice, but was absent in NK1R KO's. Finally, alcohol reward, as measured by conditioned place preference, is absent in NK1R KO's, perhaps indicating an involvement of this mechanism not only in negative but also in positive reinforcement by alcohol (Thorsell et al., submitted for publication).

Capitalizing on the availability of orally available, brain penetrant NK1R antagonists with demonstrated safety in humans, we have obtained a degree of human translation of the mouse NK1R findings. Using positron emission tomography (PET), we determined the relationship between dose and central NK1R occupancy (RO) for the high affinity NK1R antagonist LY686017. Based on these data, we were able to select a dose, 50 mg daily, which yields a >90% blockade of central NK1R's. Human activity of the antagonist was evaluated in 50 recently detoxified anxious alcohol-dependent subjects, hospitalized at the NIAAA inpatient unit throughout the duration of a 1-month experimental medicine trial. The predictive validity of individual surrogate markers is not well established, and a battery of experimental outcomes was therefore used. LY686017 produced a highly consistent profile of effects across the different outcomes. The antagonist suppressed spontaneous alcohol cravings, and had a beneficial effect on global measures of well being, in the absence of effects on general psychopathology. Toward the end of the study, we exposed subjects to a challenge intended to simulate a situation under which relapse risk is high. To achieve this, we measured craving responses to a combination of an established stress challenge (TSST) (Kirschbaum et al., 1993), and equally established alcohol-cue exposure procedure (Monti et al., 1993). Treatment with LY686017 reduced both the subjective craving response to the

combined challenge, and the concomitant cortisol response. Finally, we used fMRI to study the effects of LY686017 treatment on brain responses to standardized affective stimuli from the IAPS (Lang et al., 1995). As mentioned above, alcoholics exhibit exaggerated behavioral and brain responses to images associated with negative affect, and conversely, exhibit reduced brain responses to standard positive images (Gilman and Hommer, 2008). Brain responses in the placebo group were in agreement with those findings. In contrast, subjects treated with LY686017 had less activation to the negative images than the placebo group in several brain regions associated with emotional response to visual stimuli. In particular, the LY686017 group had less activation in the insula, as indicated above a brain region involved in craving and addictive behavior (Naqvi and Bechara, 2009). Unexpectedly, the LY686017-treated group also showed greater brain activation in the Nc. Accumbens and anterior cingulate cortex to the positive IAPS images than the placebo treated group, essentially normalizing the deficit in brain responses to positive affective stimuli otherwise found in alcoholics. Together, the attenuation of responses to negative, and restoration of responses to positive affective stimuli may reflect an overall shift in the balance between positive and negative emotionality reflected in the subjective improvement detected by the clinical ratings.

In summary, NK1R antagonism has rapidly emerged as an attractive candidate treatment mechanism in alcoholism. It remains to be determined whether its beneficial effects are exclusively related to stress mechanisms, or whether effects on acute alcohol reward also contribute. More importantly, larger clinical trials that directly assess drinking outcomes under outpatient conditions are needed to determine the clinical potential of NK1R antagonists for treatment of alcoholism. Such trials are currently underway.

5. Conclusions

Alcohol use disorders are major causes of morbidity and mortality (Ezzati et al., 2002). Although spontaneous remission occurs, classical studies show that relapse occurs within a 12-month period in almost 2/3 of clinical cases in conventional treatment programs (Hunt et al., 1971). The introduction of the competitive opioid antagonist naltrexone and the functional glutamate antagonist acamprosate provided proof of concept for pharmacotherapy of alcohol addiction, but effect sizes are limited (Bouza et al., 2004). These discoveries therefore are of exceptional conceptual importance, but have had a more limited clinical impact. Extensive unmet medical needs therefore remain in the area of alcoholism treatment. Recent research points to fundamental pharmacogenetic heterogeneity of both alcohol and naltrexone responses, indicating that outcomes can be markedly improved if naltrexone treatment is targeted to the right patient. Research further points to a critical involvement of pathologically activated stress systems in later stages of alcoholism. Several anti-stress mechanisms, exemplified by CRH1 or NK1R blockade, therefore promise to offer valuable additions to the treatment toolkit. Together, these advances may finally allow neuroscience-based treatments to make a major clinical impact.

Conflict of interest

The authors have no conflict of interest to declare.

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