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A key role for corticotropin-releasing factor in alcohol dependence

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Abstract

Recent data indicate that alcohol dependence induces long-term neuroadaptations that recruit a negative emotional state. This leads to excessive alcohol ingestion motivated by relief of negative emotionality. A key mechanism in this transition to negative reinforcement is a recruitment of corticotropin-releasing factor (CRF) signaling within the amygdala. Long term upregulation of CRF_1 receptors is observed in the amygdala following a history of dependence, and CRF antagonists selectively block emotionality, excessive alcohol drinking and stress-induced reinstatement of alcohol-seeking in post-dependent animals. Innate upregulation of CRF_1 receptor expression mimics the post-dependent phenotype, both with regard to emotional responses and ethanol self-administration. Therefore, the CRF system is emerging as a key element of the neuroadaptive changes driving alcoholism and as a major target for its treatment.

Introduction – a neuroadaptive perspective on alcohol dependence

Alcohol use accounts for 4% of global disease burden [1]. Alcohol dependence, or alcoholism, is a complex disorder in which heritable susceptibility factors contribute 50–60% of the disease risk, and interact with environmental factors to produce and maintain the disease state [2]. Alcoholism is characterized by uncontrolled heavy drinking and a chronic relapsing course [3]. Relapse, that is, return to heavy drinking after intervals of sobriety, is key to this process. Reduction of heavy drinking and relapse prevention are therefore main therapeutic objectives in alcoholism treatment.

Alcoholism evolves over 5–10 years [4]. A central thesis of this paper is that over that time, its motivational and neural substrates undergo a major shift. Early stages of excessive alcohol use are characterized by impulsive drinking, that is, drinking to intoxication in binge-like episodes, common among adolescents and young adults in industrialized countries [5,6]. This behavior is positively reinforced by pleasurable alcohol effects, and interspersed by periods of sobriety during which the individual initially returns to a neutral emotional and motivational state. During this stage, environmental stimuli become associated with pleasurable alcohol effects, setting the scene for ‘reward craving’ [7].

By contrast, following a prolonged history of dependence, low mood, elevated anxiety and increased sensitivity to stress become dominant. At this stage, alcohol use becomes primarily negatively reinforced, that is, motivated by the ability of the drug to eliminate what can

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collectively be labeled ‘a negative emotional state’. This sets the scene for craving motivated by relief produced through renewed drug intake, or ‘relief craving’ (Figure 1).

This shift is a prime example of ‘reward allostasis’, that is, establishing reward equilibrium at a new, pathological level of functioning following a prolonged period of environmental load [8–10]. The concept of ‘anti-reward’ postulates the existence of brain mechanisms that over time limit drug reward, through neuroadaptations recruited in response to repeated drug taking. Neuroadaptations can be ‘within systems’ mediating drug reward themselves, or occur through activation of systems that oppose those (‘between systems’) [11]. A key proposition of this paper is that between-systems neuroadaptations, represented by recruitment of an over-active corticotropin-releasing factor (CRF) system, mediate stress sensitivity and negative emotions in later stages of alcoholism. Understanding the pathology of this circuitry offers attractive novel targets for alcoholism treatment.

History of dependence and the post-dependent phenotype

Laboratory rodents do not voluntarily consume alcohol to intoxication, in part because of taste aversion similar to what humans experience when they first sample alcohol. Higher levels of consumption can be achieved by masking the taste of alcohol with a sweetener, which is faded out as alcohol concentrations are increased. However, even using fading procedures, rats that have not been bred for high alcohol preference will rarely consume in excess of 2 g alcohol/kg/day, and blood alcohol concentrations (BACs) required for dependence will rarely be achieved [12,13]. The predictive validity of consumption at this level for human alcohol dependence is unclear [12].

By contrast, prolonged periods of forced brain exposure to BACs commonly occurring in human alcoholism (~150–250 mg/dL) will trigger long-term neuroadaptations leading to markedly increased voluntary alcohol intake (Figure 2), and other consequences relevant for clinical alcoholism. This syndrome can collectively be labeled ‘the post-dependent state’ (Box 1). Forced intoxication can be induced by offering an alcohol-containing liquid diet as the sole source of food, or by intragastric alcohol gavage. However, alcohol vapor inhalation offers numerous practical advantages for this purpose [14]. Compared with control conditions without alcohol exposure, vapor exposure produces markedly upregulated levels of alcohol self-administration that persist long beyond acute withdrawal [15], and results in long-term dysregulation of neuronal gene expression profiles [16]. Repeated cycles of forced intoxication and withdrawal, paralleling the clinical course of alcoholism, are more effective in inducing the post-dependent state than constant exposure [16–20]. This might be due to progressively increasing activation of glutamatergic transmission over repeated withdrawals [21]. Duration of exposure is also critical, and persistent phenotypic changes are obtained after longer exposure duration [22]. Excessive self-administration or consumption induced by a history of dependence is qualitatively different from basal intake, and probably of particular relevance for human alcoholism. Some of the key evidence for this comes from studies on the CRF system reviewed below.

Box 1. The post-dependent state

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. Based on genetic susceptibility and other factors, the extent and duration of post-dependent neuroadaptations show considerable individual variability. In some individuals, the

neuroadapted, post-dependent state is hypothesized to remain indefinitely (‘once an alcoholic, always an alcoholic’), while in others it remits.

CRF, behavioral stress responses and emotionality in the post-dependent state

CRF is a 41 amino acid polypeptide with a wide distribution throughout the brain. The highest densities of CRF-positive neurons are found within the paraventricular nucleus of the hypothalamus, but CRF-positive cells 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 amygdala, and the brainstem [23,24]. CRF was discovered as the hypothalamic releasing factor for adrenocorticotropic hormone (ACTH) [23], but was subsequently also found to mediate a broad range of behavioral stress and anxiety responses [25]. CRF actions are mediated through two categories of G protein-coupled receptors, CRF_1 and CRF_2, which are primarily G_s-coupled in neurons [26]. Hypothalamic CRF neurons mediate endocrine stress responses through activation of pituitary CRF_1 receptors. By contrast, behavioral stress responses are largely mediated by extrahypothamic CRF_1 receptors, primarily in the amygdala and BNST. Effects of CRF_2 activation are less clear, but are commonly opposite to those of CRF_1 [27,28]. CRF_1 signaling mediating behavioral stress responses is quiescent under a wide range of conditions, but becomes activated in the presence of uncontrollable stress [29,30]. This illustrates the principle that neuropeptides are commonly released at high neuronal firing frequencies, acting as ‘alarm systems’ [31].

Elevated anxiety is a hallmark of alcohol withdrawal, and is CRF mediated, because it is blocked by CRF antagonism in the CeA [32,33], while CRF levels or release are increased both in CeA [34] and BNST [35] during this phase. More importantly, in animals with a history of dependence, stress sensitivity and a negative emotional state remain upregulated long after acute withdrawal has subsided. This represents a shift in responsiveness rather than a shift of baseline. No overt differences in anxiety-like behavior were observed in a standard anxiety model, the elevated plus-maze, during protracted abstinence, but when animals were challenged by restraint stress, anxiety-like behavior was markedly accentuated in the post-dependent group. This accentuated anxiety was blocked by the non-selective peptide CRF antagonist D-Phe $CRF_{12–41}$, while the antagonist was without effect in the control group that had not been previously exposed to alcohol [36]. Similarly, following a history of dependence, enhanced fear-induced suppression of behavior was found in another classical anxiety model, the Vogel conflict test, where it persisted for extensive periods of time (6–12 weeks) after exposure to alcohol. Notably, the Vogel test is inherently a stressor, because of the foot shock used to create the conflict. The increase in anxiety-like behavior in this model following a history of dependence was also fully reversed by systemic treatment with a selective CRF_1 antagonist, in this case 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-*b*]pyridazine (MTIP) [37].

Similar results have been obtained using other means to induce the post-dependent state. Rats subjected to three cycles of withdrawal from an alcohol diet, but not to a single withdrawal, showed increased anxiety-like behavior during early abstinence [38]. Although this acute withdrawal effect subsided within 48h, a remaining recruitment of systems mediating a negative emotional state was demonstrated by the observation that, following a history of three withdrawal episodes, a single further withdrawal from re-exposure to chronic ethanol led to an increased anxiogenic-like effect. In this model, administration of a CRF antagonist during the first two cycles of withdrawal blocked the increase in anxiety-like behavior [39,40]. Furthermore, rats with a history of dependence obtained through a liquid diet protocol with multiple withdrawals were behaviorally more sensitive to a preceding restraint stress. The

potentiated anxiogenic effect of this stressor was blocked in post-dependent animals by systemic administration of the CRF_1 antagonist CP-154 526 [18]. Based on these and other observations, a 'kindling/stress' hypothesis was proposed [19]. It states that repeated cycles of withdrawal drive a progression to a sensitized stress response, and that activation of CRF_1 receptors plays a major role in this process.

CRF and dependence-induced excessive drinking

Experiments with the CRF system demonstrate that excessive post-dependent self-administration or intake of alcohol is fundamentally different from basal levels. Post-dependent animals tested two hours into withdrawal exhibited markedly elevated rates of self-administration. These were consistently brought down to non-dependent levels by systemic treatment with three different non-peptide, CRF_1 selective antagonists: antalarmin, MJL-1–109–2 or R121919 (Figure 3). None of the antagonists affected self-administration in non-dependent animals [41]. In a follow-up study, the non-selective peptide CRF antagonist D-Phe $CRF_{12–41}$ microinjected into the CeA blocked excessive post-dependent self-administration rates, while microinjections into BNST or the nucleus accumbens shell were ineffective. Furthermore, CeA injections of D-Phe $CRF_{12–41}$ in animals without a history of dependence were also ineffective, once again demonstrating that the CRF system, presumably within the amygdala, is recruited to drive excessive alcohol self-administration in the post-dependent state [42].

These effects of CRF antagonism were observed during acute withdrawal, but excessive voluntary alcohol drinking and self-administration has also been found long after forced alcohol exposure [16,43,44]. D-Phe $CRF_{12–41}$ injected intracerebroventricularly blocked the increased ethanol drinking observed during both acute withdrawal and protracted abstinence, while this antagonist was inactive in animals without a history of dependence [43]. Similarly, an established procedure for dependence induction using gastric gavage [44], followed by cycles of self-administration and imposed deprivation periods, also resulted in excessive self-administration. After several weeks, the novel selective non-peptide CRF_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 [44]. In summary, recruitment of CRF signaling drives excessive alcohol self-administration and consumption in post-dependent animals, both during withdrawal and long after withdrawal has subsided.

CRF and stress-induced relapse to alcohol-seeking

Three categories of environmental stimuli are known to trigger relapse in alcohol-dependent individuals [45,46]: small, 'priming' alcohol doses; conditioned cues associated with prior availability of alcohol; and stress. The relapse process can be modeled in experimental animals using reinstatement of alcohol-seeking by any of these stimuli (Box 2). Both the non-selective D-Phe $CRF_{12–41}$ and the CRF_1 selective antagonist CP-154 526 blocked stress-induced reinstatement [47]. A subsequent study in post-dependent animals demonstrated a dissociation between cue- and stress-induced reinstatement [48]. The former, but not the latter, was blocked by the opioid receptor antagonist naltrexone, used clinically to treat alcoholism. Conversely, CRF antagonism using D-Phe $CRF_{12–41}$ blocked stress-induced reinstatement, while leaving cue-induced reinstatement unaffected. Post-dependent animals display a markedly increased sensitivity to blockade of stress-induced reinstatement by CRF antagonism [44]. The selective CRF_1 antagonist MTIP entirely blocked this behavior at 10mg/kg, a dose at which no effect was seen in animals without a history of dependence. Taken together, these data show that CRF_1 receptors mediate stress-induced reinstatement, and that a recruitment of the CRF system

in the post-dependent state renders animals preferentially sensitive to blockade of relapse-like behavior by CRF_1 antagonism.

Box 2. Animal models of alcohol consumption, self-administration and relapse

- **Voluntary alcohol consumption** can be assessed by simply providing free-choice access to two bottles, one containing an alcohol solution and the other a non-alcoholic fluid. Consumption in this type of model might, however, be driven by a variety of factors unrelated to pharmacological actions of alcohol, such as taste and calories.
- **Self-administration** approaches require an operant response, most commonly a lever-press, to be emitted for alcohol reward. These models are generally thought to gauge better the motivation to obtain alcohol. For CRF_1 antagonists, however, this distinction appears less important, and results with voluntary consumption and self-administration are the same.
- **Excessive dependence-induced versus basal consumption and self-administration** appears instead to be the key distinction for CRF_1 antagonists. The former, but not the latter, is consistently blocked by this class of compounds. Excessive self-administration and consumption can result from a prolonged history of forced brain alcohol exposure using alcohol vapor, liquid diet or gastric gavage, or from genetic selection for high alcohol preference. Forced exposure paradigms might bypass some mechanisms involved in voluntary initiation of alcohol use, but growing evidence supports their predictive validity for medications development [13].
- **Reinstatement of alcohol seeking** has in recent years been established as an animal model of relapse-like behavior. In this model, operant self-administration is first established, and during this time an association can also be formed between a discrete cue and delivery of alcohol. Lever-pressing on the alcohol lever is subsequently extinguished by removing the reinforcer. Finally, to evaluate motivation for alcohol-seeking induced by a priming dose, an alcohol-associated cue or a foot-shock stress, the respective stimulus is introduced, and lever-pressing on the previously alcohol-associated lever is measured without actually delivering alcohol [70,71].

Similarly to other CRF-mediated behavioral stress responses, adrenalectomy has no effect on stress-induced reinstatement of alcohol-seeking, demonstrating mediation by extrahypothalamic CRF systems [47]. However, the neurocircuitry through which CRF mediates reinstatement in the post-dependent state is not fully understood. A suggestive parallel might be provided by work on stress-induced reinstatement of cocaine-seeking, which showed a recruitment of CRF control over ventral tegmental area (VTA) dopamine output following a history of cocaine dependence, mediated by an upregulation of VTA glutamate release [49]. This finding maps well onto the neuronal network described for stress-induced reinstatement of cocaine-seeking [50], but it is unclear whether the circuitry is the same for reinstatement of alcohol-seeking. The α_2-adrenergic antagonist yohimbine, a pharmacological stressor that can substitute for foot-shock to reinstate alcohol-seeking [51], has recently been shown to upregulate CRF expression in CeA [52], another structure within this ‘reinstatement network’. However, to date, only one antagonist microinjection study has attempted to localize directly CRF mediation of stress-induced reinstatement of alcohol-seeking, and this study reported blockade by injections into the median raphe [53], a structure that falls outside the proposed cocaine reinstatement circuitry. Multiple CRF pathways might be involved in different addictions, and also act in concert to mediate different alcohol-related behaviors.

Neural substrates of the post-dependent behavioral phenotype – the CRF system

Recruitment of CRF 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 CRF is increased in the amygdala [34]. Presumably as a reflection of this, decreased tissue levels of CRF were seen within this structure in early withdrawal [42, 54]. Six weeks after last alcohol exposure, amygdala CRF had not only recovered, but also increased to supra-normal levels [54]. Elevated tissue content of CRF peptide in the amygdala could either reflect increased synthesis or decreased utilization. The finding of increased CRF transcript levels in the CeA during the post-dependent state supports increased synthesis in CeA in this condition [37].

A major contribution, however, comes from an upregulation of CRF_1 receptor expression and binding within the amygdala. This is consistent with the left-shifted dose–response curve for CRF_1 antagonists observed in the post-dependent state. Perhaps the best demonstration that CRF_1 upregulation produces the characteristics of the post-dependent phenotype was obtained in the genetically selected, alcohol preferring Marchigian-Sardinian Preferring (msP) rat [55]. These animals were found essentially to represent a behavioral phenocopy of post-dependent rats, with which they share increased stress sensitivity, 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 upregulation of the transcript encoding the CRF_1 receptor within the amygdala complex. This was linked to an allele at the *Crhr1* locus (that encodes the CRF_1 receptor) unique to msP rats, characterized by two single nucleotide polymorphisms (SNPs) in the promoter region. Genetic variation at the *Crhr1* locus as a susceptibility factor for excessive alcohol drinking might have parallels in humans, where a similar association was recently reported [56]. In msP rats, the selective CRF_1 antagonist antalarmin reduced alcohol self-administration to non-dependent levels and blocked stress-induced reinstatement of alcohol-seeking at doses that did not affect non-selected rats without a history of dependence [57]. 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 downregulate the receptor transcript to normal levels [58].

Following up on the msP findings, a similar upregulation of CRF_1 expression was found in genetically non-selected, post-dependent rats (Figure 4) [37]. This upregulation persisted three months after ethanol exposure, reflecting a long term neuroadaptation rather than acute withdrawal. Similar to the msP findings, receptor upregulation was most pronounced in the basolateral (BLA) and medial amygdala (MeA), and was found in CeA only to a lesser extent (W. Sommer *et al.*, unpublished). The contribution of amygdalar subnuclei to the post-dependent state remains to be established. Microinjections of a CRF antagonist in CeA blocked post-dependent excessive self-administration, but the BLA or MeA were not tested [42]. Amygdalar nuclei are interconnected, and receptors expressed in BLA or MeA neurons could be inserted into terminals in other amygdala regions. Furthermore, CRF antagonists microinjected into the brain could act at multiple, serially connected CRF sites within this structure.

The results from post-dependent and msP rats differ from those previously obtained in mutant mice lacking the CRF_1 receptor [59]. Similar to what we found in post-dependent animals with *upregulated Crhr1* expression [37], the *Crhr1* mutants showed a paradoxical, delayed increase in voluntary alcohol consumption in response to forced swim stress. This study employed constitutive mutants, in which deletion of *Crhr1* might lead to compensatory developmental effects, and altered glutamatergic function was indeed reported in this model. Also, these

animals were not dependent on alcohol. Together, msP and post-dependent data indicate that upregulation of CRF_1 receptors is sufficient to produce the post-dependent phenotype, and that blockade of this receptor can normalize post-dependent behavior.

Contribution of other neurotransmitter systems to the post-dependent phenotype

Expression of the astroglial glutamate transporter GLAST is elevated both in post-dependent rats [16] and brains of human alcoholics [60]. GLAST removes extracellular glutamate [61], and its upregulation is presumably compensatory to elevated glutamate release in the post-dependent state [21]. Given an established role for glutamate in stress pathology [62], functional glutamate antagonists might be able to suppress excessive drinking by reducing negative reinforcement by alcohol. Acamprosate, which is approved for the clinical treatment of alcoholism, might exemplify this, and suppresses post-dependent excessive drinking while leaving basal consumption in non-dependent animals unaffected [16].

Neuropeptide Y (NPY), or antagonism at presynaptic NPY–Y2 receptors that drives increased release of endogenous NPY, also selectively suppress alcohol self-administration and drinking in post-dependent animals [63–66]. Importantly, in non-dependent animals, NPY is either inactive, or can increase intake of alcohol, presumably through hypothalamic stimulation of appetite [67]. More than a decade ago, we proposed that NPY counteracts effects of CRF within the amygdala [68]. We note that the selective suppression by NPY of excessive post-dependent drinking is exactly what would be predicted by that model.

Summary and translational perspective

Recent data demonstrate a recruitment of extrahypothalamic CRF systems following a history of alcohol dependence, or owing to genetic selection for high alcohol preference. This does not necessarily confer an overt phenotype, but when faced with a stressor, individuals with a hyperactive CRF system have exaggerated emotional responses. Furthermore, either post-dependent or innate upregulation of the CRF system gives rise to excessive rates of alcohol self-administration, presumably through negative reinforcement of alcohol intake. Finally, susceptibility to stress-induced relapse-like behavior is also upregulated under these conditions. Data from post-dependent and genetically selected animals converge, and point to an upregulation of CRF_1 receptors within the amygdala as a major factor behind the behavioral phenotype described here. Together, these data predict that blocking hyperactive signaling at CRF_1 receptors in individuals with a history of dependence or innate susceptibility will inhibit heavy drinking and reduce the risk of relapse. As CRF_1 antagonism selectively blocks stress- but not alcohol-cue associated relapse, a combination of naltrexone and a CRF_1 antagonist might act synergistically to prevent relapse.

No treatments yet exist that effectively target the phenomena of negative emotional state, stress-sensitivity and relief-craving in alcoholism. Targeting pathological negative emotional states and drug-seeking motivated by relief craving is an attractive addition to the treatment armamentarium. Complying with medications that act through this kind of mechanism is inherently reinforced. The lack of effect under non-pathological conditions, combined with an ability to inhibit heavy drinking and relapse, and an attractive profile from the perspective of the patient offer considerable promise that CRF_1 antagonists might represent a major step forward for the treatment of alcoholism, and indeed, as has been suggested previously, perhaps also other addictions [69].

Outstanding questions

Recruitment of the CRF system is not the only long-term neuroadaptation that occurs with a history of dependence. A key phenomenon that seems to evolve in parallel is a recruitment of a hyperglutamatergic state, which shifts central excitation–inhibition balance towards excitation [21]. An important outstanding question is whether a link exists between neurodapatations affecting CRF and glutamate systems, or whether they independently contribute to the post-dependent phenotype.

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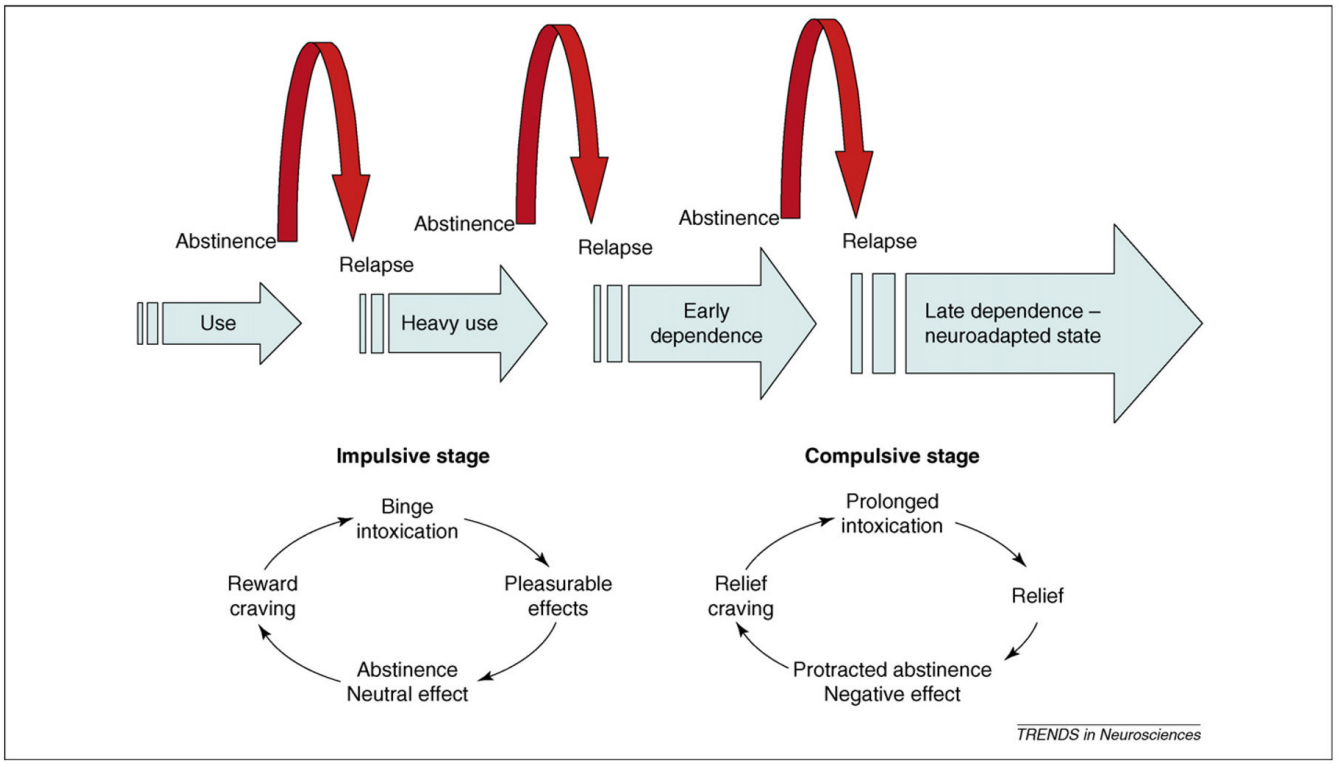

Figure 1.

The progression of alcohol dependence over time. The schematic illustrates the shift in underlying motivational mechanisms. From initial, positively reinforcing, pleasurable alcohol effects, the addictive process progresses over time to being maintained by negatively reinforcing relief from a negative emotional state. Data presented in this article suggest that neuroadaptations encompassing a recruitment of extrahypothalamic CRF systems are key to this shift.

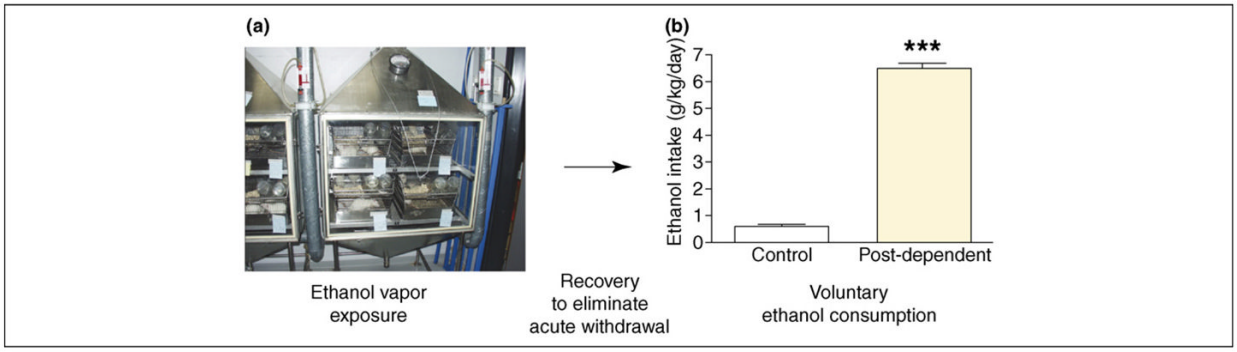

Figure 2.

Following a history of ethanol dependence induced by exposure to ethanol vapor, long-lasting neuroadaptations lead to the induction of excessive levels of voluntary alcohol consumption. **(a)** Post-dependent neuroadaptations can be induced by intermittently exposing animals to intoxicating alcohol levels over 7 weeks, and then allowing them to recover for 3 weeks to eliminate acute withdrawal. **(b)** A behavioral hallmark of the post-dependent phenotype is a marked and persistent upregulation of voluntary ethanol intake compared with animals that have been exposed to air only. Data taken from Ref. [22].

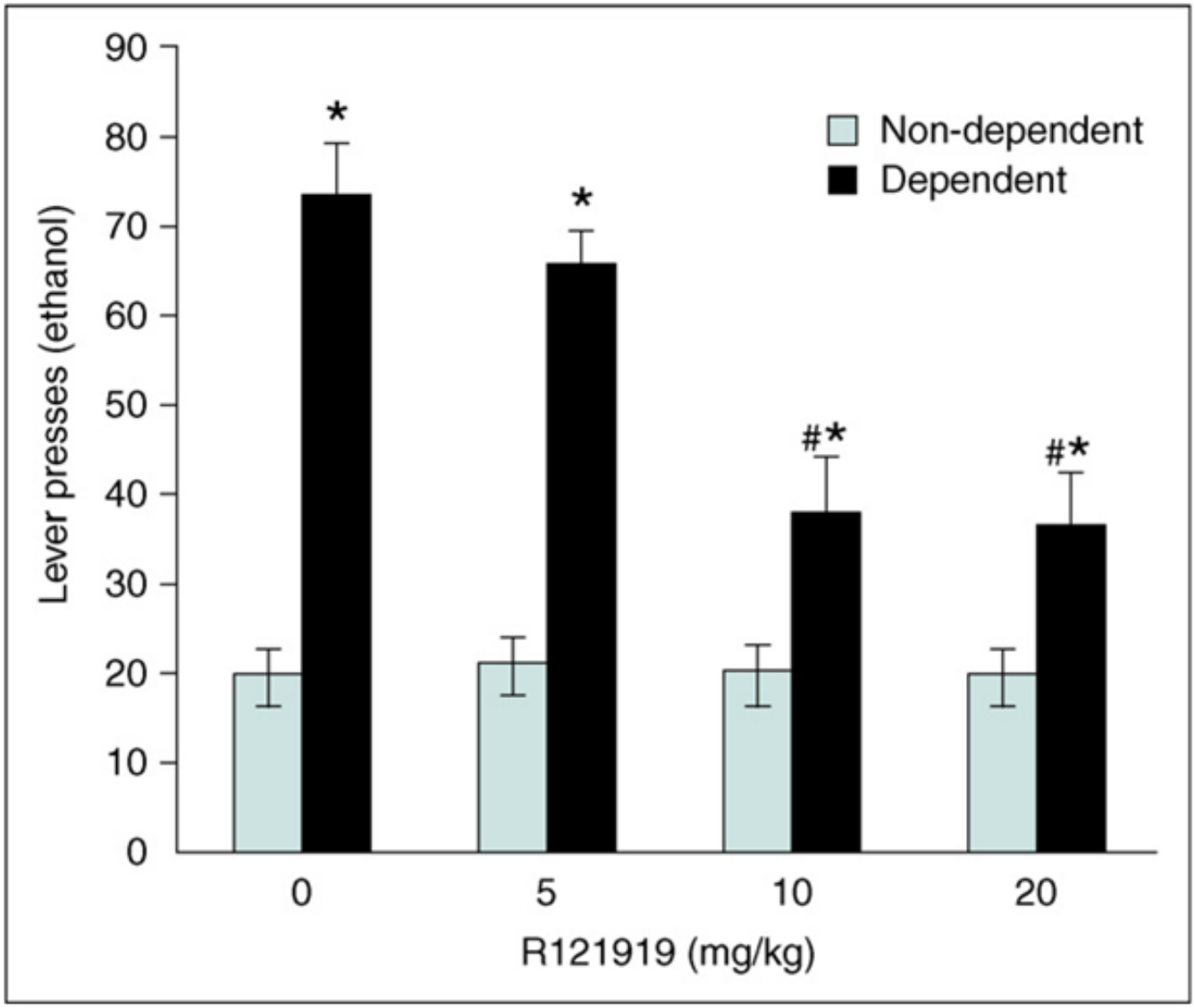

Figure 3.

A hallmark of all CRF1 receptor antagonists tested to date is their ability to block selectively excessive rates of operant alcohol self-administration, or excessive two-bottle free-choice alcohol consumption in post-dependent animals at doses that do not affect these behaviors in animals without a history of dependence [41,44]. This is illustrated here by the prototypical non-peptide CRF_1 antagonist, R121919 (*$P < 0.001$ compared with the same drug dose in non-dependent animals; #$P < 0.0001$ compared with vehicle treatment in dependent animals). Clinical development of R121919 for major depression was discontinued, but similar data have been obtained with other non-peptide CRF_1 antagonists that might have a potential for clinical development, such as antalarmin [41,57] or MTIP [44]. Redrawn, with permission, from Ref. [41].

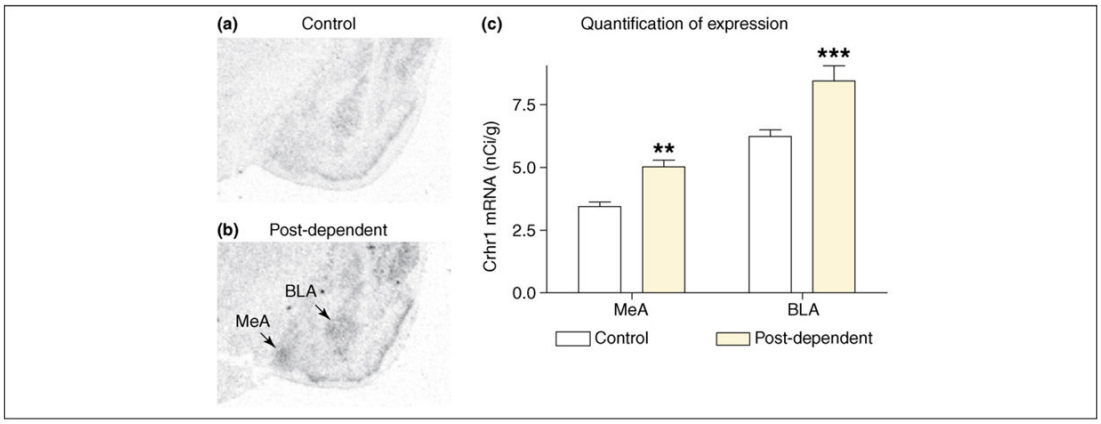

Figure 4.

A long-term upregulation of the *Crhr1* transcript, encoding the CRF_1 receptor, is found within the amygdala complex in the post-dependent state, and is likely to mediate excessive alcohol consumption. **(a,b)** Illustrative sections of (a) control rats and (b) post-dependent animals that have been intermittently exposed to intoxicating alcohol levels over 7 weeks, and then allowed to recover for 3 weeks to eliminate acute withdrawal. **(c)** Quantitative analysis of *in situ* hybridization data from the two groups. Basolateral amygdala (BLA) and medial amygdala (MeA) are particularly affected. These data parallel, and provide a biological substrate for, observations that CRF_1 receptor antagonists can block the elevated voluntary drinking and increased behavioral sensitivity to stress found in post-dependent animals. Data taken from Ref. [37].