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A role for GABA mechanisms in the motivational effects of alcohol

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Abstract

Low doses of ethanol have been hypothesized to act directly via proteins that form ligand-gated receptor channels, such as the γ-aminobutyric acid (GABA) receptor complex, to allosterically alter function, particularly in specific brain areas such as those hypothesized to be involved in ethanol reinforcement. At the pharmacological level, one can antagonize the effects of ethanol with GABA antagonists, particularly its sedative, anxiolytic-like and acute reinforcing actions. Brain sites involved in the GABAergic component of ethanol reinforcement include the ventral tegmental area, elements of the extended amygdala (including the central nucleus of the amygdala), and the globus pallidus. Chronic administration of ethanol sufficient to produce dependence and increased ethanol intake are associated with increased GABA release in the amygdala and increased sensitivity to GABA agonists. A hypothesis is proposed that GABAergic interactions with the brain stress neurotransmitter corticotropin-releasing factor in specific elements of the extended amygdala may be an important component for the motivation for excessive drinking associated with the transition from social drinking to addiction.

Keywords: Alcohol; γ-Aminobutyric acid; Extended amygdala; Dependence

1. Alcohol and GABA

Alcohol to date does not have an identified specific neurotransmitter binding site in the brain, but ethanol-receptive elements within membranes—and a protein component of neuronal membranes in particular—may provide a sensitive site for ethanol actions [1,2]. The question is how these ethanol-receptive elements convey specificity of action and how this translates into behavioral action.

Ethanol has been hypothesized to interact with a number of ligand-gated ion channels, and low doses of ethanol (10–50 mM) have been hypothesized to act directly upon proteins that form ligand-gated receptor channels such as the γ-aminobutyric acid (GABA) receptor complex, particularly in specific brain areas such as those hypothesized to be involved in ethanol reinforcement [3–8]. The in vitro actions of ethanol on the $GABA_A$ receptor are some of its most potent effects, with doses as low as 1–3 mM being effective at altering GABA-gated current measures [9]. Ethanol appears to modulate the GABA receptor complex allosterically to basically open the chloride channel and hyperpolarize cells, or at least potentiate the hyperpolarization produced by GABA. At the pharmacological level, one can antagonize the effects of ethanol with GABA antagonists. Approach avoidance behavior is reduced by ethanol [10], and ethanol produces anti-conflict actions in the social interaction test, elevated plus maze, and in operant procedures [11]. These anticonflict effects are blocked by drugs that interact with the GABA-receptor complex to decrease functional activity. The anti-conflict effect of alcohol is blocked by the GABA antagonist picrotoxin [12] but not by the benzodiazepine antagonist flumazenil [13]. Isopropylcyclophosphate, a compound that binds near or at the chloride ionophore regulated by GABA, blocks the anti-conflict effects of alcohol at low doses [14] (see Fig. 1). Low doses of benzodiazepine inverse agonists also block the anti-conflict effects of alcohol but can have anxiogenic-like effects on their own at these doses [13,15].

Systemic injections of $GABA_A$ antagonists also reverse the motor-impairing effects of ethanol [3,16]. The GABA agonist muscimol potentiated the sedative effects of ethanol, and the noncompetitive $GABA_A$ antagonist picrotoxin reduced ethanol-induced sedation [3]. Both direct and indirect GABA agonists potentiated ethanol-induced increases in the aerial righting reflex, while the competitive $GABA_A$ antagonist bicuculline reversed the effects of ethanol on the righting response [17].

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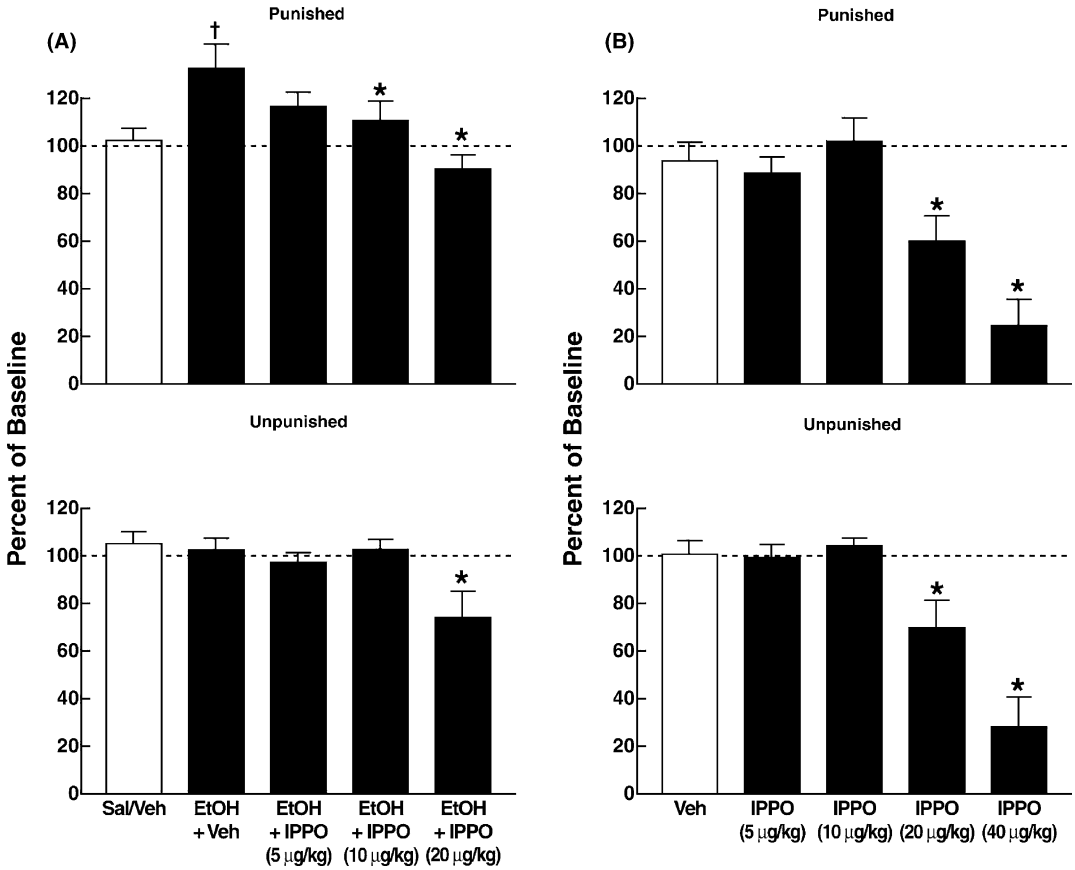

Fig. 1. (A) The effects of ethanol and three doses of isopropylbicyclophosphate (IPPO) + ethanol on responding during an operant conflict test. Ethanol produced a significant increase in responding during the conflict component (top) that was dose-dependently reversed by IPPO. Ethanol and IPPO + ethanol had no effect on reinforcers earned during the unpunished fixed-interval component (bottom) except at the highest dose of IPPO. †Significant difference from saline/vehicle group ($P < 0.05$; Newman–Keuls). *Significant difference from ethanol vehicle ($P < 0.05$; Newman–Keuls). Ethanol + 5 μg/kg IPPO was not significantly different from Ethanol + 10 μg/kg IPPO ($P > 0.05$; Newman–Keuls). (B) Effects of IPPO alone on responding during the operant conflict test. IPPO produced a dose-dependent reduction in responding in the punished and unpunished fixed-interval components. *Significant difference from the vehicle group ($P < 0.05$); Newman-Keuls). Taken with permission from [14].

2. Targets within the addiction cycle relevant for actions of GABA

The purpose of this review is to address the hypothesis that the neurotransmitter GABA in specific neurocircuits forms an important component of reinforcement mechanisms that drive substance dependence on alcohol or alcoholism. Alcoholism can be defined as a complex behavioral disorder characterized by preoccupation with obtaining alcohol and a narrowing of the behavioral repertoire toward excessive consumption (loss of control over its consumption). Alcoholism also is usually accompanied by the development of tolerance and dependence and impairment in social and occupational functioning. The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) [18] defines substance dependence on alcohol as a cluster of cognitive, behavioral and physiological symptoms, indicating that an individual continues use of alcohol despite significant alcohol-related problems and lists seven criteria that incorporate the above symptoms. For the purposes of this discussion, *substance dependence on alcohol*, as defined by the DSM-IV, will be considered to be operationally equivalent to the syndrome of *alcoholism*. It is recognized that animal models of a complete syndrome as complex as alcoholism are difficult to achieve, but validated animal models exist for many of the different components of the syndrome, providing a heuristic means by which to pursue the underlying neurobiological basis for the disorder (see Table 1).

In this context of substance dependence, there are two major sources for the reinforcing actions of ethanol. The first would be that there are obviously positive reinforcing effects of ethanol, and this psychological construct often is linked to the positive hedonic or pleasurable effects of alcohol. However, a second motivational aspect of alcoholism or substance dependence on alcohol is the negative reinforcing properties associated with relief of a negative affective state associated with alcohol dependence. The construct of negative reinforcement refers to the increase in the probability of a response by removal of a stimulus (usually aversive). Negative reinforcement in alcoholism can involve a genetic vulnerability for pathology, such as anxiety that is relieved

Table 1
Animal models for the DSM-IV criteria for alcoholism

DSM-IV criteria	Animal models
A maladaptive pattern of alcohol use, leading to clinically significant impairment or distress as occurring at any time in the same 12-month period:	
1. Need for markedly increased amounts of alcohol to achieve intoxication or desired effect; or markedly diminished effect with continued use of the same amount of alcohol	Increased ethanol intake with dependence induction
2. The characteristic withdrawal syndrome for substance; or alcohol is taken to relieve or avoid withdrawal symptoms	Increased reward thresholds and increased anxiety-like responses
3. Persistent desire or one or more unsuccessful attempts to cut down or control alcohol use	Conditioned positive reinforcing effects
4. Alcohol used in larger amounts or over a longer period that the person intended	Alcohol intake in dependent animals Alcohol Deprivation Effect
5. Important social, occupational, or recreational activities given up or reduced because of alcohol use	Choice paradigms Behavioral economics—loss of plasticity
6 A great deal of time spent in activities necessary to obtain alcohol, to use alcohol, or to recover from its effects	Alcohol self-administration during withdrawal
7. Continued alcohol use despite knowledge of having a persistent problem that is likely to be caused or exacerbated by alcohol use	Binge alcohol intake in selectively bred animal lines following alcohol deprivation

by ethanol self-administration, such as drinking to reduce the anxiety associated with a comorbid anxiety disorder. Alternatively, drinking excessively can engage the brain stress systems, and drinking may produce negative reinforcement by reducing stress responses. The combination of positive reinforcement, genetic vulnerability, psychosocial stressors, and drug-reduced stress may constitute a powerful substrate for alcohol reinforcement that may involve changes in GABAergic function that provide a key component of the motivation to seek alcohol in alcohol dependence. Although less explored to date, some of the changes in GABAergic function may persist in protracted abstinence to contribute to vulnerability to relapse.

3. Extended amygdala: a basal forebrain macrostructure as a focal point for GABAergic actions on alcohol reinforcement

The anatomical construct termed the *extended amygdala* represents a macrostructure that shares similarities in morphology, neurochemistry and connectivity, and is composed of several basal forebrain structures: the lateral and medial bed nucleus of the of the stria terminalis (BNST), the central and medial amygdala, the area termed the sublenticular substantia innominata, and a transition zone in the posterior medial part of the nucleus accumbens (e.g., shell) [19]. This system receives limbic and olfactory afferents and projects heavily to the hypothalamus and midbrain. As such, the extended amygdala links the basal forebrain to the classical reward systems of the lateral hypothalamus via the medial forebrain bundle reward system. A guiding hypothesis is that many of the neuropharmacological effects of ethanol on GABAergic function, including its rewarding and ''anxiolytic'' effects may be mediated by this circuitry, and that neuroadaptive changes in this reward circuit also may provide the motivation for excessive drinking characterized by dependence and relapse [20] (see later).

4. A role for GABA in acute ethanol reinforcement

Animal models for the acute reinforcing effects of alcohol were greatly facilitated by advances in animal models for drinking. Whereas early paradigms which assessed the reinforcing effects of ethanol typically used an oral preference paradigm where animals were allowed to drink ethanol or water, a validated operant procedure for limited access to ethanol subsequently provided a reliable procedure for measuring the motivation for drinking pharmacologically relevant doses of alcohol [21,22]. A major breakthrough in this domain was the development of a training procedure involving access to a sweetened solution and a subsequent fading in of ethanol to avoid the aversiveness of the ethanol taste. As a result, this procedure is a reliable means of measuring the reinforcing effects of ethanol, and a reliable means for exploring the neuropharmacological basis for ethanol reinforcement [23].

5. Effects of GABAergic agents on self-administration of alcohol in nondependent rats

$GABA_A$ antagonists decreased operant alcohol self-administration [24] and blocked the alcohol stimulus effects in drug discrimination [25]. Using an operant model of ethanol self-administration, pretreatment with RO 15-4513, a benzodiazepine inverse agonist, at low doses selectively decreased responding for ethanol but not for water. RO 15-4513 did not affect responding for a saccharin solution, suggesting a specific action [26] (see Fig. 2). Isopropylbicyclophosphate, a picrotoxinin site ligand, selectively decreased responding for ethanol at very low doses in alcohol-preferring, alcohol nonpreferring, and Wistar rats [26] (see Fig. 3). Chlordiazepoxide, a benzodiazepine, had no effect on responding for ethanol under these conditions. Altogether, these results suggest that acute blockade of $GABA_A$ receptor function can block the motivation for responding for ethanol, supporting the

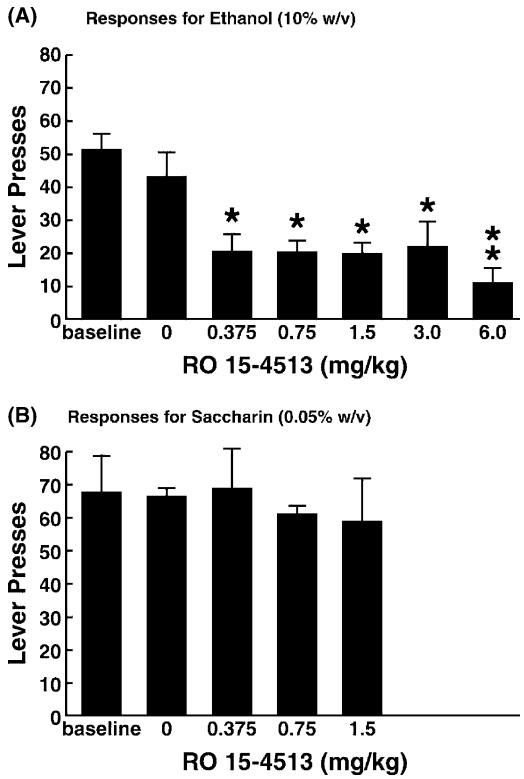

Fig. 2. (A) Effects of RO 15-4513 on responding for ethanol in a free-choice task. Responses on a fixed-ratio 1 schedule resulted in the delivery of response-contingent ethanol (10%) reinforcement. Values shown here represent the mean ± S.E.M. number of lever presses for ethanol during 30 min sessions. Asterisks indicate significant differences compared to vehicle ($^{*}P < 0.05$; $^{**}P < 0.01$; Newman–Keuls test). (B) Effects of RO 15-4513 on responding for saccharin in a free-choice saccharin self-administration task. Responses on a fixed-ratio 1 schedule resulted in the delivery of response-contingent saccharin (0.05%) reinforcement. Values shown here represent the mean ± S.E.M. number of lever presses for saccharin during 30 min sessions. Taken with permission from [26].

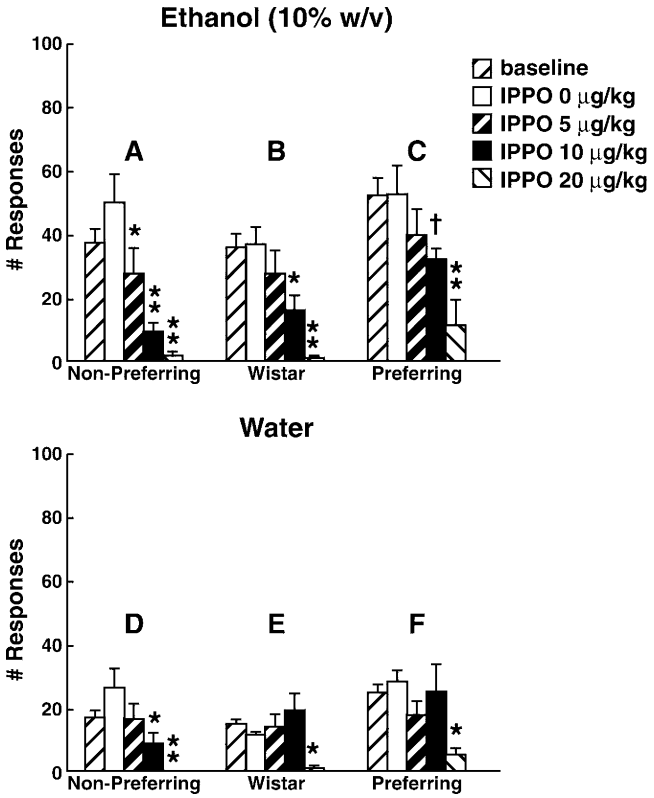

Fig. 3. Effects of isopropylbicyclophosphate (IPPO) on responding for ethanol or water in a free-choice operant task. Responses on a fixed-ratio 1 schedule resulted in the delivery of response-contingent ethanol (10%, w/v) or water reinforcement. Values shown here represent the mean ± S.E.M. number of lever presses for ethanol and water during 30 min sessions. Asterisks indicate significant differences compared to vehicle ($^{*}P < 0.05$; $^{**}P < 0.01$; Newman–Keuls test). The dagger indicates a significant difference from baseline ($^{\dagger}P < 0.05$; paired *t*-test). Taken with permission from [26].

hypothesis that activation of GABA is an important component of the acute reinforcing effects of ethanol.

$GABA_B$ receptors are metabotropic receptors that regulate potassium and calcium channels through a G-protein-mediated mechanism and exert an inhibitory cellular action in the central nervous system. A selective $GABA_B$ agonist decreases ethanol self-administration in nondependent rats [27] and the alcohol deprivation effect in alcohol preferring rats [28,29]. Several clinical studies also have shown potential efficacy of baclofen in reducing alcohol craving and ethanol withdrawal [30,31]. These studies and evidence that $GABA_B$ receptor agonists may modulate mesolimbic dopamine neurons have provided a rationale for the hypothesis that activation of $GABA_B$ receptors may decrease the reinforcing actions of ethanol [32].

In addition, a very potent GABA antagonist, SR 95531, when microinjected into the basal forebrain, significantly decreased ethanol consumption [33]. The GABA antagonist was injected bilaterally into the nucleus accumbens, BNST and central nucleus of the amygdala in rats trained to self-administer alcohol in a limited access procedure. The most sensitive site was the central nucleus of the amygdala; doses as low as 2 and 4 ng of SR 95531 when injected into the central nucleus of the amygdala decreased alcohol self-administration [33] (see Fig. 4). Using ethanol as the cue for drug discrimination, the direct GABA agonist muscimol substituted for ethanol when injected into the nucleus accumbens or core or central nucleus of the amygdala [34]. Others have observed that a mixed agonist/antagonist at the benzodiazepine site of the $GABA_A$ receptor that interacts with the α_1 subunit of the $GABA_A$ receptor can significantly decrease ethanol self-administration when injected into the central nucleus of the amygdala [35] and the ventral pallidum, an important projection of the extended amygdala [36,37].

6. Effects of GABAergic agents on self-administration of alcohol in dependent rats

The question of what circuits in the brain are involved in negative reinforcement associated with alcohol withdrawal have been focused on two dependent variables: motivational measures of withdrawal and animal models of

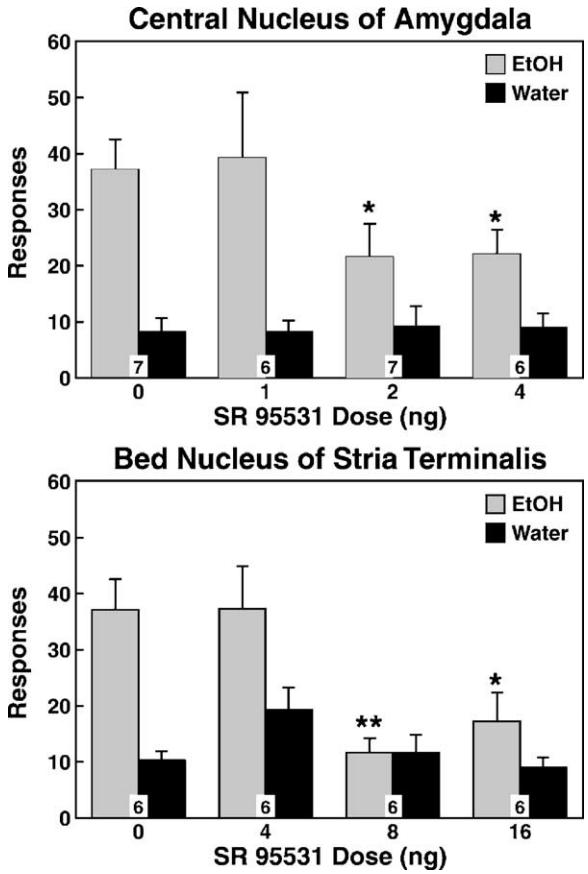

Fig. 4. The effect of SR 95531 injections into the central nucleus of the amygdala and the BNST on responding for ethanol (EtOH) and water. Data are expressed as the mean ± S.E.M. numbers of responses for ethanol and water during 30 min sessions for each injection site. Asterisks indicate significant differences from the corresponding saline control values ($^{*}P < 0.05$, $^{**}P < 0.01$ for ethanol responses). Taken with permission from [33].

excessive drinking during dependence. GABAergic mechanisms have been implicated in both domains and suggest a role for GABA in the neuroadaptations associated with the transition in humans from limited access to ethanol to chronic binging or chronic drinking on a daily basis.

Ethanol withdrawal in humans and animals is characterized by a central nervous system hyperexcitability that results in both physical and ''affective'' signs of dependence. In humans, early stages (up to 36 h) are characterized by tremor and elevated sympathetic responses including increase in heart rate, blood pressure and body temperature. Such physical signs are accompanied by insomnia, anxiety, anorexia and dysphoria. Late stages of withdrawal, if untreated (which is now rare), can include more severe tremor, sympathetic responses, anxiety and delirium tremens. In animals, physical signs include tremor, loss of the ventromedial distal flexion reflex, weight loss, and audiogenic- or stress-induced seizures. More ''affective'' signs have included increased responsiveness of acoustic startle [38], disruption of operant behavior [39], stimulus generalization to an anxiogenic drug [40], and increased sensitivity in behavioral tests of anxiety and stress such as the elevated plus maze [39]. The time course of ethanol withdrawal in the rat ranges up to 7 days, with peak effects manifesting at 8–24 h [41], and the severity of withdrawal is related to the blood ethanol levels attained, the duration of the treatment, and prior history of ethanol withdrawal [41–43].

While physical or somatic signs of alcohol withdrawal are useful markers for the general hyperexcitability of the alcohol dependent state, the more ''motivational'' signs have more relevance to the negative reinforcement construct described above. Startle amplitude is enhanced during ethanol withdrawal [38,44] with maximal effects observed during the first 4–8 h. Ethanol withdrawal also decreases prepulse inhibition [38], a measure that may reflect increases in distractibility and that has been shown to be impaired in psychosis [45]. Ethanol withdrawal also can disrupt ongoing behavior, decreasing operant responding on a mixed fixed-ratio/fixed-interval schedule of food reinforcement [46]. Ethanol withdrawal also produces a pronounced anxiogenic-like response in animal models of anxiety. Rats exposed to a liquid diet of ethanol for 2–3 weeks and tested 8 h after withdrawal showed an ''anxiogenic-like'' response on the elevated plus maze [39], including reductions in both the percentage time spent on, and the percentage number of entries onto, the open arms of the elevated plus maze.

Finally, rats can be trained to discriminate central nervous system stimulants/convulsants from saline, and the stimulus properties of the stimulant/convulsant is generalized to the stimulus properties of ethanol withdrawal [40]. When ethanol is administered by gavage or by liquid diet, the animals selected a pentylenetetrazol (a $GABA_A$ receptor antagonist and prototypical anxiogenic drug) lever before the onset of overt physical signs of ethanol withdrawal.

Studies with pharmacological agonists and antagonists have implicated GABA systems in both the physical/somatic and the ''affective'' or more specifically the anxiogenic-like effects of ethanol withdrawal. GABA agonists decrease central nervous system hyperexcitability during ethanol withdrawal and decrease ethanol withdrawal-induced convulsions [17,47]. GABA antagonists exacerbate many of the symptoms of ethanol withdrawal [48], and the partial inverse benzodiazepine agonist RO 15-4513 has been shown to increase the incidence of seizures during ethanol withdrawal [49].

GABA also has been implicated in more ''affective'' measures of ethanol withdrawal. As described above, using a drug discrimination procedure, ethanol withdrawal as a stimulus produces stimulus characteristics similar to injection of pentylenetetrazol, an anxiogenic drug [40]. This pentylenetetrazol-like interoceptive stimulus produced by ethanol withdrawal is potentiated by bicuculline and picrotoxin, suggesting that the anxiogenic-like response produced by ethanol withdrawal may be related to an ethanol-induced alteration in the function of the GABA-benzodia-

zepine ionophore complex [50]. Also, the benzodiazepine antagonist flumazenil (RO 15-1788) reversed the anxiogenic effects of ethanol withdrawal using the social interaction test in rats [51]. The flumazenil effect appeared to be very long-lasting, suggesting some possible long-term interaction with the benzodiazepine ionophore complex or an endogenous ligand acting upon this complex.

Another neurotransmitter system hypothesized to be involved in the "affective" aspects of ethanol withdrawal is the brain stress neurotransmitter corticotropin-releasing factor (CRF). CRF itself has anxiogenic-like actions, and CRF antagonists have the opposite effects, reversing many behavioral responses to stress [52]. CRF antagonists also reverse the anxiogenic-like response of rats during ethanol withdrawal [39]. These actions of CRF antagonists have been linked to elements of the extended amygdala in that microinjection of the CRF antagonist into the central nucleus of the amygdala also reversed the anxiogenic-like responses of ethanol withdrawal at a dose which is ineffective when administered intraventricularly [53] (see Fig. 5). There also is evidence that chronic ethanol increases the sensitivity of rats to the locomotor-activating effects of CRF [54]. Also, hypothalamic CRF was increased in rats that showed a high preference for ethanol in a free-choice situation [55]. Extracellular levels of CRF are increased in the central nucleus of the amygdala during acute ethanol withdrawal [56], and even more compelling, a competitive CRF antagonist injected intracerebroventricularly reversed the excessive drinking of ethanol associated with ethanol withdrawal and protracted abstinence [57]. These results suggest that selective extrahypothalamic CRF systems, in addition to the classic hypothalamic–pituitary–adrenal axis, may be altered during the ethanol dependence cycle, and this could be reflected in an overactivity during withdrawal.

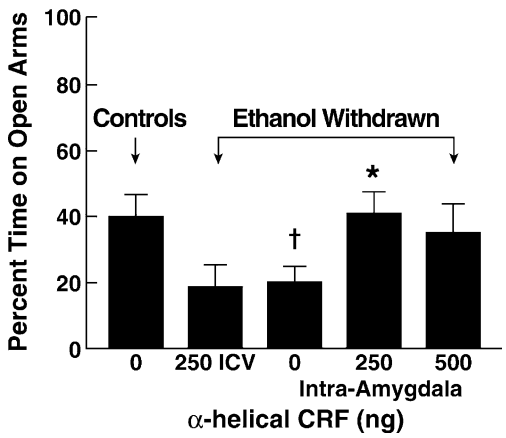

Fig. 5. Effects of microinfusion of α-helical-CRF_{9-41} into the central nucleus of the amygdala and i.c.v. administered α-helical-CRF_{9-41} in the elevated plus maze during ethanol withdrawal. Data are expressed as mean ± S.E.M. of percent time exploring the open arms. The asterisk indicates a significant difference compared to vehicle treatment ($^{*}P < 0.05$). The dagger indicates a significant difference compared to pair-fed controls ($^{\dagger}P < 0.05$). Taken with permission from [53].

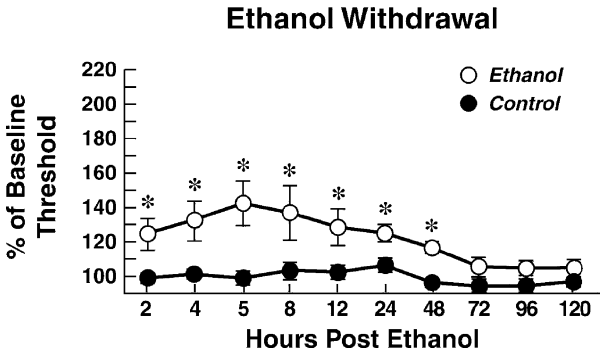

Fig. 6. Time-dependent elevation of intracranial self-stimulation thresholds during ethanol withdrawal. Mean blood alcohol levels achieved were 197.29 mg%. Data are expressed as mean ± S.E.M. percentage of baseline threshold. Asterisks indicate thresholds that were significantly elevated above control levels at 2–48 h post-ethanol ($^{*}P < 0.05$). Open circles indicate the control condition. Closed circles indicate the ethanol withdrawal condition. Taken with permission from [58].

To address the question of changes in the reward system associated with drug dependence and alcohol dependence, measures of reward function following chronic drug exposure were performed using the technique of intracranial self-stimulation. Acute administration of many drugs of abuse lower thresholds for brain stimulation reward [58]. However, following chronic drug administration, thresholds are augmented or increased, which means there is a decrease in reward during acute withdrawal (i.e. more electrical current is required to activate the neurons of the medial forebrain bundle). During acute ethanol withdrawal there is a prolonged increase in reward thresholds that lasts up to 72 h [58] (see Fig. 6). Thus, one can hypothesize that the function of the medial forebrain bundle has been compromised by chronic administration.

GABAergic drugs can modify reward thresholds, suggesting that GABA mechanisms may modulate reward and may be involved in the changes in reward associated with acute withdrawal. Both $GABA_B$ agonists and antagonists can increase brain stimulation reward thresholds suggesting a complex interaction with the reward system and GABA function, possibly reflecting differential effects at pre- and post-synaptic receptors [59]. These changes in reward function are accompanied by changes in neurochemical systems within the extended amygdala that include decreases in neurotransmitter function implicated in the acute reinforcing effects of alcohol (e.g., GABAergic systems). Using an animal model of ethanol self-administration in dependent rats, a GABA agonist was shown to selectively decrease ethanol self-administration in dependent but not nondependent rats [60].

Rats were trained to lever press for 10% ethanol using the saccharin fade-out procedure. Half of the rats were put into the ethanol vapor chambers for dependence induction and half were placed in control air chambers. After 2 weeks of vapor exposure, the rats were withdrawn every 4 days for a total of five tests. Immediately upon being removed from the vapor the rats were placed in the operant boxes and

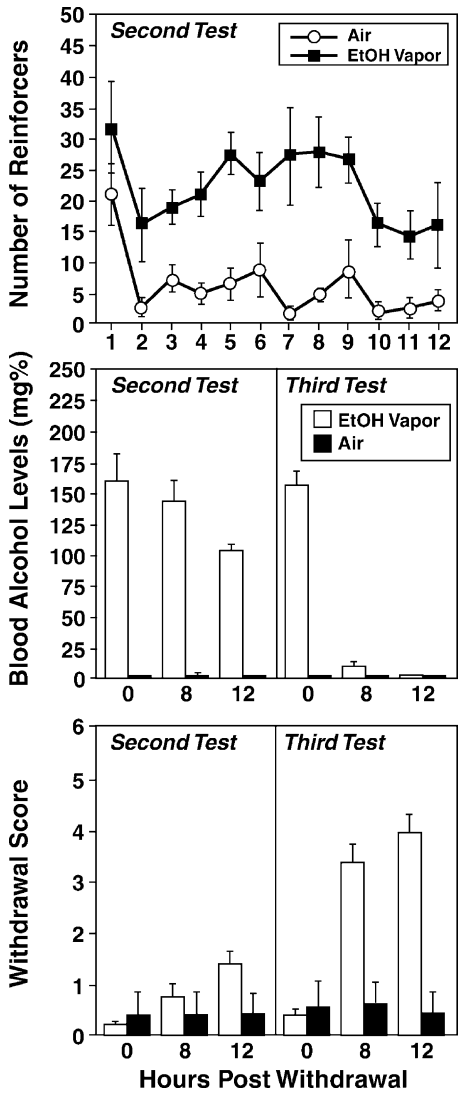

Fig. 7. Operant responding for ethanol (EtOH) across a 12 h test period by air-exposed and ethanol vapor-exposed rats (top). In addition, blood alcohol levels (middle) and ethanol withdrawal severity (bottom) obtained during test 2 (while rats were allowed access to ethanol in the operant boxes) and test 3 (while in home cages) are shown. Data are expressed as means ± S.E.M. Taken with permission from [60].

allowed to respond for ethanol and water across the entire 12 h period of withdrawal. Dependent rats responded to a greater degree than nondependent controls and in fact maintained blood alcohol levels above 100 mg% over the 12 h period and as a result did not show the withdrawal signs present in dependent rats not allowed to respond for ethanol during the withdrawal phase [60] (see Fig. 7). Subsequent work showed that responding across repeated withdrawal sessions increased, suggesting that the rats learn to respond in a manner which controls their blood alcohol level and minimizes or avoids withdrawal discomfort [61].

The enhanced ethanol self-administration during acute withdrawal was reduced dose-dependently by intracerebral pretreatment of the GABA agonist muscimol into the central nucleus of the amygdala [60] (see Fig. 8). Muscimol significantly decreased responding for ethanol in alcohol-dependent animals but had no effect in nondependent controls in either extended or limited access tests. These results suggest that increases in GABA activity in the amygdala selectively decrease alcohol consumption in dependent rats, and suggests that GABAergic systems in the amygdala may change with the development of dependence.

Changes in GABAergic function in the amygdala during the development of dependence to alcohol is supported by biochemical, electrophysiological and pharmacological studies. Pharmacological studies with GABA agonists show decreased sensitivity to GABA activation after chronic ethanol [62,63], and GABA agonists are well known to block acute withdrawal from alcohol [17,47].

Chronic administration of ethanol can decrease GABA-mediated responses in cerebral cortex [64,65], and nucleus accumbens [66], and the functional activity of benzodiazepine inverse agonists are enhanced in chronic ethanol-exposed animals [67,68]. Decreased muscimol-stimulated chloride uptake in the cortex of ethanol-exposed animals also suggests a decreased sensitivity of this system during the development of dependence [64,69,70].

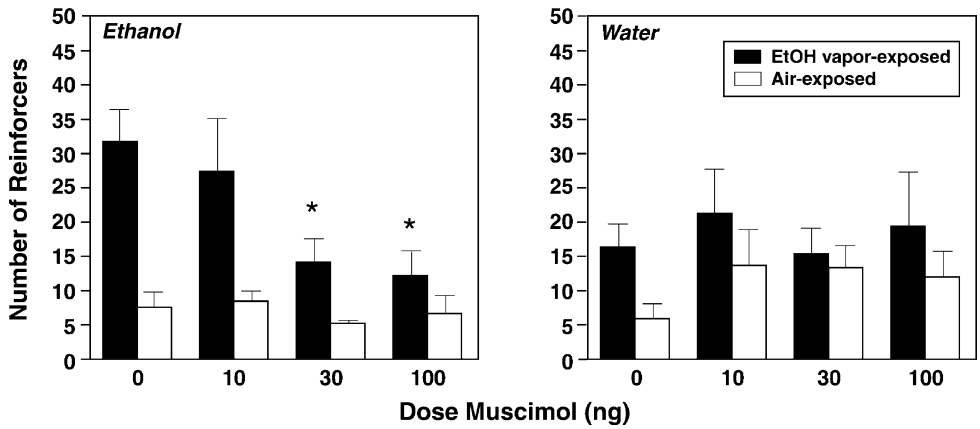

Fig. 8. Operant responding for ethanol and water in ethanol vapor-exposed and air-exposed rats after intra-amygdala administration of muscimol, a $GABA_A$ receptor agonist. Data are the means ± S.E.M. of hours 7 and 8 post-withdrawal of 12 h withdrawal sessions where animals had access to ethanol self-administration for all 12 h. Asterisks (*) indicate significant difference ($P < 0.05$). Modified with permission from [60].

Chronic ethanol increases the release of GABA into the central nucleus of the amygdala and facilitates GABAergic neurotransmission [71]. Using an in vitro slice preparation from the central nucleus of the amygdala, acute superfusion of ethanol (11–66 mM) enhanced $GABA_A$ inhibitory postsynaptic potential/current (IPSP/C), with recovery on washout. The ethanol effect on IPSP/Cs in chronically ethanol-exposed animals was quantitatively similar to that in neurons from naive rats, suggesting a lack of tolerance. In chronic ethanol-exposed rats, the overall amplitude of evoked IPSP/Cs was larger, spontaneous IPSP/C activity was increased, and baseline paired-pulse facilitation of IPSP/Cs was decreased compared to naive rats, suggesting that evoked GABA release was increased. In vivo administration of ethanol (0.1, 0.3, 1.0 M) via a microdialysis probe produced a dose-dependent increase of GABA release in central nucleus of the amygdala dialysates of both chronic ethanol treatment and naive rats.

Such functional changes in the GABA system are also accompanied by changes in the expression of specific subunits of the $GABA_A$ receptor with decreases in the α_1 subunit and increases in the α_4 and α_6 subunit [72]. These results suggest that changes in GABA neurotransmission and/or the GABA/benzodiazepine ionophore complex may contribute to the development of ethanol dependence. The neurocircuitry site of action for these changes remains unknown, but the differential distribution of the α_1 and α_2 subunits of the $GABA_A$ receptor within the extended amygdala with strong expression of the α_1 in the medial amygdala, and strong expression of the α_2 subunit in the central division of the extended amygdala may have some relevance [73]. In addition, the specific brain sites critical for mediating these GABAergic effects on specific aspects of dependence, notably motivationally relevant aspects, remain to be determined.

CRF/GABA interactions have been shown in other brain areas and support a complex interaction that is dependent on the brain site. In the paraventricular nucleus of the hypothalamus, it appears that CRF neurons are under tonic inhibitory control of an intrinsic GABAergic circuit [74]. GABAergic receptors are localized on CRF neurons in the paraventricular nucleus [75], and GABA may be colocalized in some CRF neurons of the parvocellular division of the paraventricular nucleus [76]. In contrast, in the frontal cortex, CRF may control GABAergic activity through an interaction with serotonin [77], and such an interaction may be of relevance to pathological changes in the frontal cortex associated with CRF in suicide victims [78]. There is also evidence showing that CRF controls GABA release in the ventral pallidum [79]. Together these results suggest that GABA may control CRF activity within local circuits in the paraventricular nucleus of the hypothalamus, but in the basal forebrain CRF activity may control GABAergic activity as seen in the amygdala.

The specific neural substrates for the anxiogenic effects of ethanol withdrawal are likely to involve the same neural elements of the extended amygdala that mediate the acute reinforcing effects of ethanol and may involve specific changes in both the GABA systems and systems such as CRF classically associated with behavioral responses to

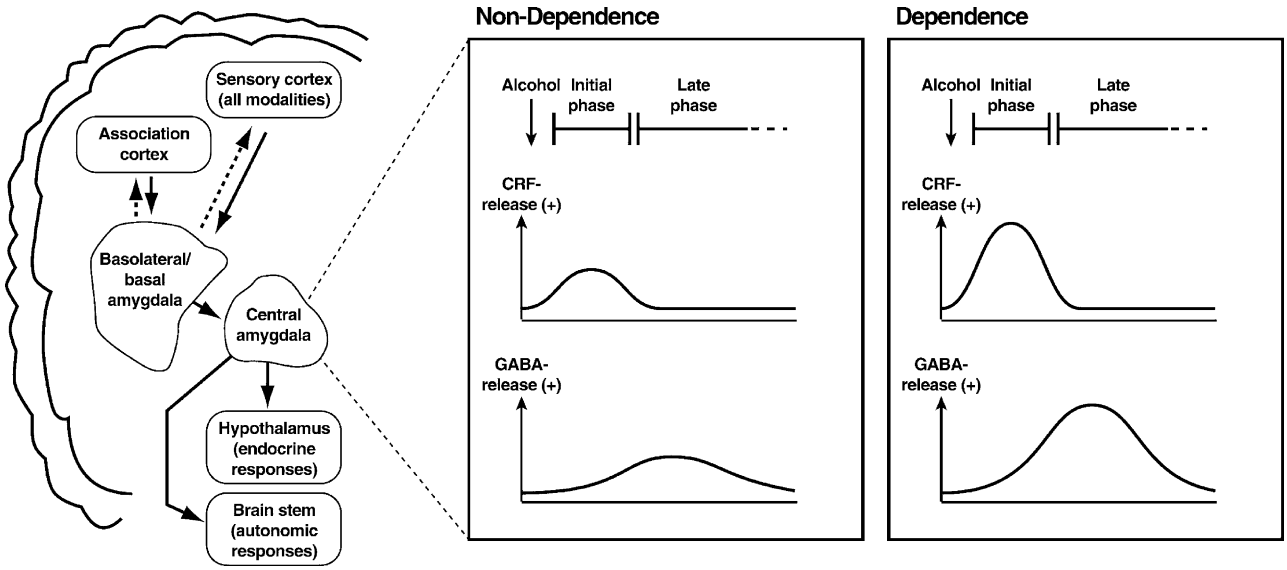

Fig. 9. Hypothetical interaction of CRF and GABA in the amygdala contributing to the reinforcing effects of ethanol with a focus on the anxiolytic-like effects of acute alcohol administration and the anxiogenic-like effects of ethanol withdrawal. The amygdala has long been considered part of an integration of sensory and associative cortical information transduced to an output that activates endocrine, autonomic and behavioral responses to stressors. Alcohol stimulates CRF release during the initial phase which in turn activates GABA release. The increase in CRF release gets progressively greater as dependence develops and is followed by a concomitant increase in GABA release to help buffer the physiological effects of the CRF activation. Somewhat paradoxically, the increased CRF availiability conveys an increased sensitivity to CRF antagonists but an increased sensitivity to GABA agonists, suggesting differential post-synaptic adapatations to functional activation of these systems. Regardless of the exact mechanism, CRF and GABA are hypothesized to interact to produce some of the motivation for the excessive drinking of alcohol in the dependent state. Derived from a conceptually similar figure involving neuropeptide Y from [82].

stressors. Particularly intriguing are recent data suggesting that chronic alcohol exposure increases the release of GABA in the amygdala and that GABA may interact with the brain stress systems in the amygdala. Indeed, GABA and CRF are co-localized and co-synthesized in the central extended amygdala [80]. In nondependent mice, ethanol (44 mM) in an amygdala slice preparation increased the activity of GABAergic neurotransmission as measured by GABA IPSPs [81]. Blockade of the CRF_1 receptor or molecular genetic knockout of the CRF_1 receptor blocked the GABAergic facilitation produce by ethanol or CRF in this model. Chronic ethanol increases both CRF and GABA release in the amygdala, suggesting that the two systems are intimately linked in the development of dependence. One possibility is that the stress-like aversive effects of CRF are modulated by the subsequent activation of GABA neurotransmission (see Fig. 9).

Several pharmacological observations may support this conceptual framework. CRF antagonists are effective in blocking the excessive drinking associated with ethanol dependence [57], and as noted above, a GABA agonist injected into the amygdala has the same effect. Neither treatment is effective in blocking ethanol self-administration in nondependent animals. Similarly, both GABA agonists and CRF antagonists block the anxiogenic-like effects of ethanol withdrawal (see above and [53]). Both GABA and CRF are co-synthesized in the same neurons in the central nucleus of the amygdala and the lateral BNST [80]. Together these results suggest that there is an important ethanol/CRF/GABAergic interaction in the extended amygdala that may be of motivational significance for the transition from controlled, nondependent drinking to excessive drinking associated with the development of alcohol abuse and alcoholism in vulnerable individuals.

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