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Factors Mediating Alcohol Craving and Relapse: Stress, Compulsivity, and Genetics

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Abstract

This article represents the proceedings of a symposium at the 2004 annual meeting of the International Society for Biomedical Research on Alcoholism in Heidelberg, Germany. The symposium was organized by Zachary A. Rodd and Giancarlo Colombo. The presentations were (1) Pharmacological reversal of cycled withdrawal-sensitized or stress-sensitized withdrawal anxiety and enhanced ethanol drinking, by Darin J. Knapp and George R. Breese, (2) Alcohol craving and relapse in rats genetically selected for high alcohol preference, by Zachary A. Rodd and Richard L. Bell, (3) Exposure to stress increases dopaminergic burst firing in awake rats, by Kristin Anstrom and Donald J. Woodward, (4) Involvement of cannabinoid CB1 and GABA_B receptors in the control of relapse-like drinking in alcohol-preferring Sardinian alcohol-preferring rats by Giancarlo Colombo and Salvatore Serra, and (5) Stress-induced ethanol drinking in CB1^{-/-}, POMC, and PENK knockout mice, by Ildiko Racz and Andreas Zimmer.

Keywords

Alcohol Relapse; Alcohol Withdrawal; Alcohol Reinforcement; Alcohol Pharmacotherapeutics

Animal models are invaluable tools for elucidating the normal and abnormal functions of the human nervous system and behavior. The ability of any animal model to depict aspects of the complex processes that underlie human behavior is predicated on that model being a relevant analog of the human condition. A number of animal models, examining different facets of alcohol-related behaviors, have been constructed to study genetic and neurobiological factors underlying alcoholism and alcohol abuse.

In humans, relapse behavior is a ubiquitous problem for individuals “recovering” from alcoholism, since at least 60 to 80% of abstinent alcoholics will relapse during their lifetime (Barrick & Connors, 2002; Chiauuzzi, 1991; Jaffe, 2002; Weiss et al., 2001). Although a number of criteria for relapse have been put forth, (Chiauuzzi, 1991), the primary criterion holds that a return to levels of ethanol consumption equal to or greater than that observed prior to “abstinence” constitutes a relapse. Because alcohol abuse has been difficult to model in

nonhuman species, it is not surprising that there are a limited number of animal models purported to mimic relapse to high alcohol drinking behavior.

In rodents, recent research has indicated a number of progressions in the attempt to model alcoholism. Specifically, relapse drinking (binge-drinking) can be modeled in rodents through manipulations of access to ethanol, the alcohol-deprivation effect (ADE). The ADE is defined as a temporary increase in the ratio of alcohol/total fluid intake and voluntary intake of EtOH solutions over baseline drinking conditions when EtOH is reinstated after a period of alcohol deprivation (Sinclair and Senter, 1967). The ADE is a phenomenon that has been observed in rats (McKinzie et al., 1998; Rodd-Henricks et al., 2000a; Sinclair and Senter, 1967), mice (Salimov et al., 1993), monkeys (Kornet et al., 1990; Sinclair, 1971), and humans (Burish et al., 1981; Mello and Mendelson, 1972). Furthermore, recent experiments have examined the effects of exposure to repeated periods of alcohol abstinence or repeated alcohol withdrawals on alcohol drinking in rodents (Holter et al., 1998; Rodd-Henricks et al., 2002a, 2002b; Rodd et al., 2003).

Alcohol seeking, a presumed animal correlate to alcohol craving in humans, has been modeled in a number of paradigms. Drug-Induced Reinstatement is a procedure in which the ability of noncontingent priming injections of previously self-administered drugs to reinitiate lever responding after extinction training is tested (Gerber and Stretch, 1975; Stretch et al., 1971). Cue-Induced Reinstatement is an experimental paradigm that assesses the ability of environmental cues to reinitiate lever responding after extinction training (Ciccocioppo et al., 2002, 2004; Katner et al., 1999; Katner and Weiss, 1999). Pavlovian Spontaneous Recovery (PSR) is an experimental technique in which animals intrinsically reinstate response-contingent behaviors for a previously obtainable reinforcer after extinction and a period of rest.

The intent of the symposium was to present recent findings examining various aspects of alcohol relapse drinking and craving-like behaviors in rodents. Each report examined a unique facet of alcoholism modeled through an animal system, with an attempt to gain understanding of the phenomenon and perhaps to elucidate potential pharmacotherapeutics for the treatment of alcoholism.

PHARMACOLOGICAL REVERSAL OF CYCLED WITHDRAWAL-SENSITIZED OR STRESS-SENSITIZED WITHDRAWAL ANXIETY AND ENHANCED ETHANOL DRINKING

Darin J. Knapp and George R. Breese

These investigations focused on conditions that may link stress, anxiety, and relapse based on the premise that alcoholism proceeds from phases of abusive drinking to eventual relapse more readily when cycles of stress and/or ethanol intake were involved (Breese et al., 2004a, 2004b; Le et al., 2000; Rodd-Henricks et al., 2001; Sinha, 2001; Spanagel and Höltér, 1999; Weiss et al., 2001). Previously, evidence showed that three five-day cycles of exposure to an ethanol diet followed by two days of withdrawal persistently sensitized anxiety-like behavior in Sprague-Dawley rats observed in the social interaction and elevated-plus maze tests (Overstreet et al., 2002). Such an effect was not found after exposure to similar amounts of ethanol in a continuous fashion over the same period of time. This phenomenon was reminiscent of the repeated withdrawal-sensitization of seizures investigated previously (Ballenger and Post, 1978; Becker and Hale, 1993; McCown and Breese, 1990) and was mimicked or blocked by repeatedly exposing animals to selective pharmacological agents (Knapp et al., 2005; Overstreet et al., 2003, 2004). This sensitization was also mimicked by repeatedly stressing the animals before or during ethanol exposure, and this action was attenuated by pharmacologically pretreating the stress episodes (Breese et al., 2004a). Further,

treating the withdrawals limited the ability of a future stress episode to elicit the sensitized anxiety-like response (Breese et al., in press).

Related data demonstrated that alcohol-preferring P rats had a lower threshold for elicitation of this sensitization and that relatively short-term (five-day) cycles of voluntary ethanol intake and withdrawal could also elicit an alcohol deprivation effect (ADE) in P rats (5 to 6 g/kg per day versus 3.5 g/kg per day in noncycled rats) (Breese et al., 2004a). This effect persisted over 24 to 48 hours and was not accompanied by a change in anxiety-like behavior. P rats that were exposed to restraint stress during the first and second deprivation/withdrawal periods exhibited a comparable early (24 to 48 hours) deprivation-induced increase in alcohol intake. However, the drinking persisted for more days in these stressed animals such that their drinking averaged approximately 8 g/kg per day over the five-day comparison period. Furthermore, these stressed, ethanol-deprived P rats exhibited anxiety-like behavior in the social interaction test on withdrawal from the voluntary alcohol drinking after the third cycle. Thus, stress increased both voluntary drinking and withdrawal-induced anxietylike behavior in the P rats. Furthermore, when multiple stress exposures (60-minute restraint) were substituted for ethanol withdrawal experiences in Sprague-Dawley or P rats, a sensitized anxiety-like behavioral response occurred after a future withdrawal, even though the animals had not had multiple withdrawals. This effect did not appear to be related to circulating levels of corticosterone, nor were there stress effects in non-ethanol exposed animals.

In pharmacological experiments, many agents with actions on the GABA/benzodiazepine, 5-HT, or CRF (corticotropin-releasing factor) systems attenuated the development of the multiple withdrawal-sensitized response. For example, intraperitoneal administration of the nonpeptide CRF type-1 receptor antagonists CRA1000 (3 mg/kg) or CP-154,526 (10 mg/kg) or 5-HT_{2C} receptor inverse agonist SB243213 (3 mg/kg) or antagonist SB242084 (3 mg/kg) during early withdrawals blocked the sensitized anxietylike behavior that was assessed during a future untreated withdrawal (Overstreet et al., 2004). Actions of other agents such as acamprosate (300 mg/kg), flumazenil (5 mg/kg), alphaxalone (9 mg/kg) diazepam (1 mg/kg) (Knapp et al., 2005), the GABA_B receptor agonist baclofen (2.5 mg/kg), and the 5-HT_{2C} receptor antagonist ritanserin (3 mg/kg) were also found to exert such effects, but the glutamate modulating agents lamotrigine (30 mg/kg), ifenprodil (10 mg/kg), MK-801 (0.3 mg/kg), the peripheral benzodiazepine receptor antagonist PK11195 (10 mg/kg), the relatively specific 5-HT_{2A} receptor antagonist ketan-serin (3 mg/kg), and the 5-HT_{1A} receptor antagonist WAY 100635 (3 mg/kg) were not active.

Other data show that the anxiety-like behavior of ethanol withdrawal can be pharmacologically sensitized and that select sites in brain appear to be important to the actions of specific agents. For example, repeated treatments of the animals with the benzodiazepine receptor inverse agonist DMCM (0.5 mg/kg IP or 0.3 µg/1 µl intra-amygdalarly) during the two weeks before ethanol exposure worsened the withdrawal from a future relatively short-term chronic ethanol exposure. Similarly, administration of the 5-HT_{2C} receptor inverse agonist Ro600175 (0.3 mg/kg) or the peptide CRF exerted similar sensitizing effects. In other central administration studies, repeated infusions of buspirone into the raphe (0.5 µg/0.5 µl) or the 5-HT_{2C} receptor inverse agonist SB243213 (3 µg/side, 1 µl) bilaterally into the amygdala were active, but reversing the treatments in the two regions was not. Intra-amygdala flumazenil (5 µg/ side, 1 µl) had comparable prophylactic actions.

When given before restraint stress in Sprague-Dawley rats, CRA1000, flumazenil, and buspirone, blocked the interaction of stress and ethanol withdrawal on anxiety-like behavior (Breese et al., 2004a). Similarly, in P rats repeatedly stressed during deprivation periods after cycles of voluntarily ethanol drinking, intraperitoneal administration of the atypical neuroleptic olanzapine (5 mg/kg), flumazenil, CP154,526, and buspirone blocked the sensitized anxiety-

like behavior, whereas naloxone (20 mg/kg), Haldol (0.5 mg/kg), and SB242084 did not. Further, treating the withdrawal periods with intraperitoneal buspirone, flumazenil, or CP-154,526 prevented a future stress from eliciting a sensitized anxiety response in Sprague-Dawley rats. Finally, pharmacological treatment of the stresses revealed that flumazenil, CRA1000, CP154,526, buspirone, naloxone (20 mg/kg), SB242084, or olanzapine reduced the stress enhancement of the ADE without appearing to significantly modify the ADE itself.

These and other reports (Rodd-Henricks et al., 2001; Spanagel and Höltér, 1999; Weiss et al., 2001) highlight the importance of the cycling pattern of ethanol exposure in the severity of withdrawal-type symptoms and the contribution of concurrent stress, receptor subtype, and animal strain in the manifestation of multiple withdrawal effects and stress on anxiety-like behavior and voluntary ethanol intake. Based on the allostasis (Koob and Le Moal, 2001; Koob, 2003) and stress/kindling (Breese et al., 2004b) models of alcoholic progression, it would appear imperative to treat withdrawal as well as stress periods, regardless of the temporal relation of these two critical variables. Finally, the relatively wide range of pharmacologically relevant targets during these early phases of adaptation to repeated ethanol exposure and stress emphasizes the potential importance of treating subjects early and possibly with more than one agent.

EXPOSURE TO STRESS INCREASES DOPAMINERGIC BURST FIRING IN AWAKE RATS

Kristin K. Anstrom and Donald J. Woodward

Alterations in dopaminergic neurotransmission, particularly activity of ventral tegmental area dopamine neurons, have been implicated in reward-related behaviors, including alcohol seeking and ingestion. In anesthetized animals, dopaminergic neuronal activity patterns consist of a combination of tonic and phasic burst firing modes (Grace, 1991) hypothesized to be regulated by dissociable circuit mechanisms (West et al., 2003). Tonic, steady-state extracellular dopamine levels are extremely stable, subject to slow rises and declines, and can be measured by dialysis. Circuit mechanisms hypothesized to regulate tonic dopamine levels include glutamatergic stimulation of synaptic dopamine release (Grace, 1991; West et al., 2003; Zigmond et al., 1998) as well as GABAergic regulation of dopaminergic population responses, or the number of active dopamine neurons (Floresco et al., 2003). Both systemic alcohol and self-administered alcohol cause increases in tonic dopamine in the striatum (Weiss et al., 1993) and have been shown to increase firing rate of dopamine neurons in vitro (Brodie et al., 1999).

In comparison, burst activity in dopamine neurons, defined as transient rapid firing of dopamine cell bodies, produces an enhanced release of intrasynaptic dopamine as compared with regular firing at the same rate (Gonon, 1988; Wightman and Zimmerman, 1990), but whether or not burst firing contributes to changes in tonic dopamine levels is debatable (Floresco et al., 2003; Phillips and Wightman, 2004). Burst firing is dependent on afferent stimulation from areas such as the prefrontal cortex (Kitai et al., 1999), subthalamic nucleus (Lokwan et al., 1999), and the pedunclopontine nucleus (Floresco et al., 2003), and transient effluxes of extracellular dopamine release due to burst firing can be measured in afferent areas by techniques such as fast-scan electrovoltametry.

Electrophysiological studies in awake animals have shown that salient events critical to learning, reward processing, and attentional selection elicit transient burst activity in dopamine cell bodies (Freeman et al., 1985; Horvitz et al., 1997; Schultz et al., 1993). In anesthetized animals, there is evidence that exposure to psychoactive drugs increases impulse activity (Marinelli et al., 2003) and that higher levels of dopaminergic burst firing may predict higher

initial levels of responding for cocaine (Marinelli et al., 2000). It is unclear, however, how phasic activity contributes to sustained firing patterns in awake animals, how behaviorally relevant conditions change sustained impulse activity levels on a real-time basis, and if levels of dopaminergic burst firing can predict alcohol-reinforced response patterns. Identification of real-time changes in dopaminergic firing patterns in relation to specific phases of alcohol-seeking or alcohol-taking behavior would thus give insight in to large-scale circuit mechanisms underlying discrete phases of these behaviors.

Acute stress is known to precipitate relapse in alcoholics and can reinstate alcohol-seeking behavior in animal models of relapse (Le and Shaham, 2002). One of the physiological responses to acute stress is a slow, steady increase in extracellular dopamine in the striatum (Imperato et al., 1992; Keller et al., 1988) and the prefrontal cortex (Abercrombie et al., 1989; Morrow et al., 1999). Whether or not stress increases activity in dopaminergic neurons is unclear. Stress activates *c-fos* expression in the ventral tegmental area (Morrow et al., 2000; Redmond et al., 2002), but others have found that stress does not increase dopaminergic activity in awake cats (Strecker and Jacobs, 1985). Recent developments in multiunit recording techniques have allowed us to record chronic activity patterns in single dopaminergic neurons across sequential days. By determining if and how dopaminergic firing patterns change on a real-time basis in models of stress, we will be able to identify which circuits may modulate dopaminergic neurotransmission and contribute to chronic behavioral states leading to relapse.

INVOLVEMENT OF CANNABINOID CB1 AND GABAB RECEPTORS IN THE CONTROL OF RELAPSE-LIKE DRINKING IN SARDINIAN ALCOHOL-PREFERRING RATS

Giancarlo Colombo and Salvatore Serra

Accumulating lines of experimental evidence indicate that the prototypic GABA_B receptor agonist, baclofen, reduces alcohol consumption under different experimental procedures (Colombo et al., 2004a). These data suggest that the GABA_B receptor may be considered a novel player among the receptor systems controlling different aspects of alcohol drinking behavior, including alcohol consumption, alcohol relapse, and alcohol appetitive properties.

Recent studies have investigated the effect of baclofen on the so-called alcohol deprivation effect (ADE), i.e., the transient increase in alcohol intake that occurs in several animal species after a period of abstinence from alcohol. This phenomenon has been proposed to model the loss of control over alcohol and the episodes of alcohol relapse in human alcoholics (McBride et al., 2002). These experiments were conducted by using the Sardinian alcohol-preferring (sP) rats, one of the few rat lines selectively bred worldwide for high alcohol preference and consumption. Rats of the sP line appear to constitute a proper animal model for pharmacological investigations on ADE, since they have been found to display a pronounced ADE during the first hour of reaccess to alcohol after a period of deprivation from alcohol (Agabio et al., 2000; Serra et al., 2003); further, ADE in sP rats has been found to be reduced by naltrexone (this laboratory, unpublished results), a drug that has some efficacy in reducing the likelihood of relapses in alcoholics (Streeton and Whelan, 2001), providing evidence of the predictive value of this animal model for the human pathology.

The first experiment (Colombo et al., 2003) evaluated the effect of baclofen on ADE in sP rats offered a choice between water and a single alcohol concentration (i.e., the standard concentration of 10%, vol/vol). Specifically, adult male sP rats were individually housed and offered alcohol and water under the standard, home cage two-bottle choice with unlimited access for eight consecutive weeks (consuming an average of approximately 6 g/kg per day alcohol). Subsequently, rats were divided into two groups (matched for alcohol intake over the

last seven days): One group was deprived of alcohol for 14 consecutive days, during which water was the sole fluid available (alcohol-deprived rats); the second group continued to have unlimited access to alcohol and water (alcohol-nondeprived rats). At the end of the deprivation phase, 30 minutes before lights-off, rats of both groups (alcohol-deprived and alcohol-nondeprived) were further divided into four subgroups ($n = 7$ to 8) and acutely injected with 0, 1, 1.7, and 3 mg/kg baclofen (intra-peritoneal). Alcohol was represented at lights-off, and its consumption was recorded 60 minutes later. Standard rat chow was available throughout the study.

Alcohol intake was higher, by approximately 50%, in vehicle-treated, alcohol-deprived rats than in vehicle-treated, alcohol-nondeprived rats, indicative of the development of a marked ADE. This increase in alcohol intake was prevented by baclofen administration; indeed, at all doses, baclofen resulted in a virtually complete suppression of the extra intake of alcohol produced by alcohol deprivation. Importantly, no dose of baclofen affected water and food intake, leading us to exclude that the action on ADE was secondary to its muscle-relaxant and sedative effects. Accordingly, a complementary experiment found that the doses of baclofen that suppressed ADE did not affect any measure of horizontal and vertical motor activities in alcohol-consuming sP rats tested in an open-field arena.

Subsequently, this laboratory investigated the effect of baclofen on the development of ADE when sP rats had access to multiple alcohol concentrations. A previous study characterized the behavior of sP rats exposed to this procedure (Serra et al., 2003). In that study, rats were initially exposed, for eight consecutive weeks, to the choice of four bottles containing 10, 20, and 30% (vol/vol) alcohol and water, respectively. Comparable amounts of alcohol (expressed in grams per kilogram of pure alcohol) were consumed daily from each bottle. After two weeks of alcohol deprivation, a robust ADE developed. Its magnitude (in terms of extra intake of alcohol) and duration were similar to those previously observed in sP rats given the single 10% alcohol solution. However, a clear shift in preference developed toward the two highest alcohol concentrations (20 and 30%), with negligible intakes from the 10% alcohol solution. ADE can be considered as an increased demand for alcohol by the “deprived” subject; consistently with this view, in the above study, the increased demand for alcohol occurred through two mechanisms: 1) augmentation of the amount of alcohol consumed and 2) selection of the alcohol concentrations (20 and 30%), which, because of a more rapid absorption of alcohol, would give faster psychopharmacological effects.

In the experiment testing baclofen, adult male sP rats were individually housed and offered alcohol (10, 20, and 30%, vol/vol) and water under the four-bottle choice with unlimited access for eight consecutive weeks (consuming an average of approximately 6 g/kg per day alcohol). Rats were then divided into two groups (matched for alcohol intake over the last seven days): One group was deprived of alcohol for 14 consecutive days, during which water was the sole fluid available (alcohol-deprived rats); the second group continued to have unlimited access to the three alcohol solutions and water throughout the study (alcohol-nondeprived rats). At the end of the deprivation phase, 30 minutes before lights-off, rats of both groups (alcohol-deprived and alcohol-nondeprived) were further divided into four subgroups ($n = 7$ to 8 in both experiments) and acutely treated with 0, 1, and 3 mg/kg baclofen i.p. Alcohol was represented at lights-off, and its consumption was recorded 60 minutes later. Standard rat chow was available throughout the study.

Alcohol intake (expressed as the sum of alcohol consumed from each of the three bottles) in vehicle-treated, alcohol-deprived rats was markedly higher than in vehicle-treated alcohol-nondeprived rats, indicating the development of ADE also under this procedure. The magnitude of this increase in alcohol intake averaged approximately 50%, being comparable to that

previously observed under the single-concentration procedure. As expected, both doses of baclofen completely suppressed the extra amount of alcohol consumed after the deprivation.

When the data on alcohol intake from each bottle were analyzed, vehicle-treated, alcohol-nondeprived rats resulted as having consumed comparable quantities of alcohol from each of the three alcohol solutions; in contrast, and in agreement with the initial observation (Serra et al., 2003), vehicle-treated alcohol-deprived rats displayed a clear preference (>80%) for the 30% alcohol solution. Interestingly, injection of both 1 and 3 mg/kg baclofen completely abolished this shift in preference.

Taken together, the results of these experiments indicate that the acute administration of baclofen suppressed both the quantitative (amount of alcohol consumed) and qualitative (preference for the most concentrated solutions) aspects of the increased demand for alcohol associated with alcohol deprivation in sP rats. These results also suggest an involvement of the GABA_B receptor in the neural substrate mediating ADE in sP rats.

Finally, because of the predictive validity of ADE as an experimental model of alcohol relapse, the results of the present study suggest that baclofen may prove to be of some efficacy in preventing relapses in human alcoholics. The results of preliminary, clinical surveys (Addolorato et al., 2002; Flannery et al., 2004) apparently support this hypothesis and suggest that baclofen may constitute a novel medication for alcoholism.

STRESS-INDUCED ETHANOL DRINKING IN CB1^{-/-}, POMC, AND PENK KNOCKOUT MICE

Ildiko Racz and Andreas Zimmer

Stress is a part of our everyday life, and each of us responds to it in a different way. Some individuals excel under stress, whereas others suffer from it. Clearly, stress can contribute to the development of psychiatric disorders, including drug addiction. Indeed, there is a substantial body of evidence demonstrating that stressful environment or lifestyle increases the probability of alcohol misuse in humans and relapse in abstinent alcoholics (Brady and Sonne, 1999).

Recently, our laboratory and others have used genetic mouse models to study the role of neuromodulators in drug-related behaviors that are influenced by stress. The endocannabinoids are lipid signaling molecules that bind and activate the neuronal CB1 cannabinoid receptor. Pharmacological studies suggested that the behavioral effects of alcohol, including alcohol drinking and alcohol preference, are modulated through the endocannabinoid signaling system (Hungund and Basavarajappa, 2004). For example, chronic ethanol treatment increases the synthesis of endocannabinoids and downregulates brain CB1 receptors (Basavarajappa and Hungund, 2002). Blockade of the CB₁ receptor with SR 141716A reduced ethanol consumption in rats and mice (Colombo et al., 2004b; Freedland et al., 2001).

We have studied the role of the endogenous cannabinoid system as a potential mediator of stress-related ethanol drinking in CB1 receptor mutant mice (CB1^{-/-}). Acute behavioral responses to ethanol were not changed in the absence of CB1 receptors. Also, there was no difference in ethanol preference in a two-bottle choice model between wild-type and mutant animals under low stress conditions, although females displayed a higher preference for alcohol in both genotypes. When CB1^{+/+} animals were exposed to a mild foot shock stressor or to a swim stress, they showed a temporary increase in ethanol preference. A social stressor (exposure to an unfamiliar animal) led to a decreased ethanol preference. Most strikingly, none of these stressors had any effect on ethanol consumption in CB1^{-/-} animals, although other physiological stress responses (e.g., *c-fos* induction) were similar in CB1^{-/-} and CB1^{+/+} mice.

We also evaluated somatic symptoms of ethanol withdrawal in animals when their access to ethanol was suddenly disrupted after several weeks of continuous ethanol consumption. Indeed, we observed strong withdrawal symptoms such as increased anxiety, handling-induced convulsions, and hypermotility in CB1^{+/+} animals. CB1^{-/-} mice, in stark contrast, showed no somatic withdrawal symptoms. Together, these results clearly point to an important role of the endocannabinoid system in stress-induced alcohol consumption and in the manifestation of alcohol dependence.

The endogenous opioid system has also been implicated in the modulation of alcohol-induced behaviors and alcohol addiction (de Waele and Gianoulakis, 1993; Kiefer et al., 2002; Oswald and Wand, 2004; Patel and Pohorecky, 1989). In fact, the opioid receptor antagonist naloxone is already in clinical use for relapse prevention in abstinent alcoholics.

We therefore applied the same behavioral protocols to study ethanol drinking behavior in endorphin-deficient POMC^{-/-} and enkephalin-deficient PENK^{-/-} mice. Our preliminary results indicate that both strains have lower preference for alcohol compared with wild-type mice, thus supporting a function of these opioid peptides in ethanol reward. We also found evidence to suggest that the lack of endorphin affected the stress modulation of alcohol consumption. Surprisingly, the effect was different in the two sexes: We found a decrease in alcohol preference in males and a significant increase in females. Interestingly, sex differences in ethanol consumption after chronic stress were also found in humans (Richman et al., 2004).

Our findings clearly indicate that the endocannabinoid and opioid systems play important and distinct roles in the modulation of several aspects that have been implicated in alcohol drinking and addiction. A further evaluation of CB1 antagonists for the therapy of alcohol addiction, perhaps in a combination with other drugs acting on opioid receptors, seems warranted.

ALCOHOL CRAVING AND RELAPSE IN RATS GENETICALLY SELECTED FOR HIGH ALCOHOL PREFERENCE

Zachary A. Rodd and Richard L. Bell

Before the late 1980s, the majority of researchers considered extinction, the loss of instrumentally learned behavioral responses or the loss of a Pavlovian conditioned response, as “unlearning,” or the destruction of old learning/memory (McClelland and Rumelhart, 1985; Rescorla and Wagner, 1972). Although this hypothesis may be intuitive, the past 20 years of research have indicated that extinction is not unlearning (Bouton, 1988, 2000, 2002, 2004; Bouton and Swartzentruber, 1991; Deleater, 2004; Falls, 1998; Rescorla, 2001). The current view of extinction is that it is not a process to destroy first-learned information but is a process of obtaining new learning (Bouton, 2002, 2004; Deleater, 2004; Rescorla, 2001). The results of numerous studies have indicated that after extinction training, both the first-learned and second-learned signals are present and that the signal or action is ambiguous (Bouton, 2002, 2004; Rescorla, 2001). The ambiguous quality of the signal/action after extinction is defined by the context.

There are at least four learning phenomena indicating that extinction does not produce unlearning (Bouton, 2002). The most common example of the retention of first-learned signals is the drug-reinstatement of responding model. US-induced (unconditioned stimulus) reinstatement (recovery of a behavior that occurs after extinction training when the animal is noncontingently administered the US) has been used to examine seeking behaviors for various drugs of abuse (Katz and Higgins, 2003; Shaham et al., 2003). Extinction training in a novel context results in the renewal phenomenon [a recovery of extinguished behavior(s) when reintroduced into the original context]. Reacquisition (the fast, often rapid, recovery of

responding when the CS (conditioned stimulus) is paired with the US again) indicates that the first-learned signals are maintained after extinction. Additionally, reacquisition testing has shown the presence of both first- and second-learned signals. In multiple experiments, the existence of second-learned signals is indicated, since discriminative stimuli paired with extinction training can reduce the rate of reacquisition (Bouton, 1988; Carlton et al., 1996; Hart et al., 1995; Rickers and Bouton, 1989). Extinction learning (second-learned) persists for an extremely long time, since cues associated with extinction training can alter reacquired operant responding for months after extinction (Brooks et al., 2001). The last phenomenon to indicate that extinction does not produce unlearning is spontaneous recovery (Pavlovian spontaneous recovery, PSR).

PSR is defined as a recovery of responding, in the absence of the previously trained reward, which is observed after a period of rest after extinction (Domjan and Burkard, 1982; Macintosh, 1977). Conceptually, PSR is a unique phenomenon in that it is time-dependent. Although this may intuitively lead to the assumption that PSR responding is the product of “forgetting about extinction,” research has indicated that this is not the case. The majority of researchers hold that PSR occurs because there is a temporal context to extinction training. Specifically, as time increases between extinction training, there is a shift in the ambiguity framework between first- and second-learned signals (Bouton, 1988; Estes, 1955; Spear, 1978). This stance is supported by research that has shown that retrieval cues for both first- and second-learned signals can alter PSR responding (Brooks, 2000; Brooks and Bouton, 1993; Brooks et al., 1999). Thus, the PSR phenomenon can be viewed as a shift from expressing second-learned signals to first-learned signals without an elimination of either learning. The expression of a PSR is directly correlated to reward saliency (Macintosh, 1977; Robbins, 1990), contextual cues associated with first-learned signals, and the amount of first- and second-learned associations (Brooks, 2000). In general, the PSR phenomenon has been asserted to be the result of an intrinsic shift away from the recent extinction (second-) learning to the initial reinforced learning responses, which reflects an intrinsic motivation to obtain the previously administered reward (Bouton, 2002, 2004; Rescorla, 2001). Therefore, the PSR model may represent a unique paradigm to study craving-like behaviors.

The first experiment from our laboratory examining the PSR phenomenon was used to determine the long-term effects of periadolescent EtOH drinking in alcohol-preferring (P) rats on alcohol seeking in adulthood (Rodd-Henricks et al., 2002a, 2002b). Briefly, P rats were given 30 days of access to 15% EtOH and water or water only during periadolescence (postnatal days 30 to 60) or adulthood (greater than postnatal day 110) followed by two weeks of water only for both groups. The results indicated that periadolescent alcohol consumption increased the rate of acquisition of oral operant self-administration, decreased the rate of extinction, and increased alcohol-seeking behaviors (PSR). In contrast, 30 days of alcohol consumption during adulthood did not alter responding during any test conditions. Compared with the periadolescent alcohol-naïve group, periadolescent EtOH drinking enhanced the expression of an EtOH PSR in P rats (Rodd-Henricks et al., 2002a). Additionally, priming amounts of EtOH (Rodd-Henricks et al., 2002a) or the presence of an EtOH odor (Rodd-Henricks et al., 2002b) increased the expression of a PSR, but these effects were greater in P rats that consumed EtOH during periadolescence. In humans, adolescent alcohol drinking may have enduring consequences, as suggested by the association of early onset of alcohol and drug abuse with increased risk for later drug-related problems, including alcoholism (Anthony and Petronis, 1995; Chou and Pickering, 1992).

Recently, we conducted a preliminary study to examine the effects of repeated alcohol deprivation on extinction responding and expression of PSR in adult P male rats. Briefly, P rats were allowed to respond for 15% EtOH and water on a concurrent FR5-FR1 schedule of reinforcement for six weeks; they were randomly assigned to one of four groups ($n = 6/\text{group}$).

The groups were never deprived (two groups), once deprived, or repeatedly deprived (four deprivations). The two nondeprived groups consisted of a group that was maintained on an FR5 schedule of reinforcement for EtOH, and a group that had the response requirement increase to an FR10. The total amount of operant chamber access to EtOH was held constant across all groups, so that the nondeprived animals were given 12 weeks and one day access to EtOH before undergoing seven consecutive extinction sessions. The once-deprived group received 12 weeks of daily access, two weeks of deprivation, and a single reexposure session before the seven consecutive extinction sessions. The repeatedly deprived group was given six weeks' access, two weeks of deprivation—two weeks of reexposure, for a total of four deprivation cycles (12 weeks of total EtOH access); after the fourth deprivation, the P rats were given one reexposure session before the seven consecutive extinction sessions. After extinction training, rats were given a two-week home cage period and then reintroduced into the operant environment in the absence of EtOH and water. During the one-session reinstatement, P rats in the deprived groups showed increased responding on the EtOH lever, which was further enhanced by repeated deprivations. A single deprivation slightly retarded extinguishing the responses on the EtOH lever during extinction training, whereas repeated deprivations had a profound effect on retarding extinction training. Additionally, nondeprived rats maintained at an FR5 schedule of reinforcement for EtOH responded more during the second extinction session than rats increased to an FR10. Thus, there was evidence for a negative contrast effect for EtOH extinction responding in P rats, and that elevated responding during extinction training is not dependent on experience with an elevated level of responding before extinction. Moreover, compared with the nondeprived and once-deprived groups, the rats that underwent multiple deprivations increased the magnitude and duration of responding during the PSR test sessions. The nondeprived groups (FR5 and FR10) displayed comparable levels of responding during the initial PSR test session (72 ± 10 and 89 ± 7). Thus, high level of responding during PSR testing is not dependent on prior experience with a high level of responding during maintenance. Similarly, past research has indicated that repeat deprivations, or exposure to repeated alcohol withdrawals, increase the breakpoint ratio during progressive ratio testing (Coccocioppo et al., 2003; Rodd et al., 2003). These data sets indicate that the reward value is increased after deprivation. Overall, data from multiple research studies suggest that neuronal alterations are occurring as a result of repeated cycles of EtOH access and deprivation that increase measures of alcohol reinforcement and alcohol seeking. It would be important to understand the neurobiological mechanisms underlying these neuronal alterations and to develop strategies to counter these changes.

SUMMARY

Overall, the presentations reported recent findings examining alcohol relapse drinking and alcohol seeking. A general finding is that repeated exposure to periods of alcohol deprivations or withdrawal periods increase ethanol drinking and other alcohol behaviors. Additionally, exposure to stressors may facilitate alcohol relapse drinking or act to alter alcohol drinking similar to exposure to repeated alcohol deprivations or withdrawals. Furthermore, the presentations highlighted potential pharmacotherapeutics for the treatment of alcoholism while indicating some of the neurotransmitter systems involved in alcohol relapse drinking and alcohol-seeking (craving) behaviors.

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