Published in final edited form as:

Alcohol Clin Exp Res. 2007 November ; 31(11): 1811–1819.

Dose- and time-dependent expression of anxiogenic-like behavior in the elevated plus-maze during withdrawal from acute and repeated intermittent ethanol intoxication in rats

Zhongqi Zhang, M.D., M.S., Andrew C. Morse, Ph.D., George F. Koob, Ph.D., and Gery Schulteis, Ph.D.

From the Department of Anesthesiology, UC San Diego School of Medicine and VA San Diego Healthcare System, San Diego, CA (ZZ, ACM, GS) and Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA (ACM, GFK, GS)

Abstract

Background—Withdrawal from acute bolus intraperitoneal injection of high doses of ethanol elicits anxiogenic-like behavior (e.g. Doremus et al., 2003; Gauvin et al., 1989, 1992) and conditioned place aversion (Morse et al., 2000). More recently we demonstrated that withdrawal from a single moderate dose of ethanol (2.0 g/kg) is accompanied by elevations in brain reward thresholds, and that repeated intermittent treatment with this dose results in a significant potentiation of reward deficit (Schulteis and Liu, 2006).

Methods—In the current study, the time- and dose-dependent emergence of anxiogenic-like behavior was measured in the elevated plus maze at various times (3–24 hr) after acute or three daily intraperitoneal (IP) injections of ethanol (1.0, 2.0, or 3.0 g/kg). Rats receiving daily handling for two days, and a single five min opportunity to explore the maze on a third day were divided into one of several treatment protocols: 1) NAIVE conditions: vehicle IP on all three days; 2) ACUTE conditions: vehicle on the first two days, ethanol on the third day; or 3) REPEAT conditions: ethanol on all three days.

Results—ACUTE ethanol elicited reduced exploration of the open arms of the elevated plus maze in a dose- and time-dependent fashion: 1.0 g/kg failed to elicit any significant effects, whereas 2.0 and 3.0 g/kg ethanol elicited a significant anxiogenic-like response at 6 hr and 9–12 hr post-injection, respectively. REPEAT treatment was still without effect at any time point tested following 1.0 g/kg ethanol, but extended the time course of anxiogenic-like behavior after treatment with either 2.0 or 3.0 g/kg doses. REPEAT treatment with 2.0 and 3.0 g/kg ethanol also produced significant hypoactivity in the maze at some time points post-injection.

Conclusions—Withdrawal from a single exposure to ethanol produces transient but significant anxiogenic-like behavior, and repeated intermittent bouts of intoxication result in a significant extension of the duration of effect. The rapid emergence and progression of negative emotional signs of withdrawal may be a significant factor in determining susceptibility to transition from casual drinking to loss of control and escalating patterns of consumption that result in alcoholism.

Keywords

Acute Withdrawal; Dependence; Abstinence; Anxiety

Alcohol dependence is characterized by an abstinence syndrome in which withdrawal symptoms resulting from CNS hyperexcitability emerge in a time-dependent fashion after cessation of drinking (Edwards, 1990; Metten and Crabbe, 1996; Mendelson and Mello, 1979; Schuckit, 1995; Schuckit et al., 1995). In addition to autonomic and somatic symptoms such as hyperventilation, tachycardia, hyperthermia, nausea, tremors and convulsions, the withdrawal syndrome also includes emotional or affective symptoms such as anxiety, restlessness, hyperirritability, and depressed mood/dysphoria (Bokstrom et al., 1989; Bokstrom et al., 1991; Roelofs, 1985; Roelofs and Dikkenberg, 1987). These negative emotional components of withdrawal, anxiety in particular, have been correlated with relapse following prolonged periods of abstinence (Annis et al., 1998; De Soto et al., 1989; Hershon, 1977; Miller and Harris, 2000; Willinger et al., 2002). For example, one study indicated that alcoholics are more than 3 times as likely to report drinking to terminate feelings of anxiety or depressed mood as they are to drink to alleviate physical withdrawal symptoms (Hershon, 1977), and a more recent study indicates that alcoholics report negative emotional states as the most common reason for relapse to drinking (Annis et al., 1998).

The somatic and affective manifestations of ethanol withdrawal become more severe with chronic use but may be observed after a single exposure to ethanol. For example, acute withdrawal or “hangover” from a single episode of ethanol intoxication may include somatic/autonomic symptoms of nausea, diarrhea, tremor, and headache, as well as emotional symptoms such as anxiety, guilt, and depression/dysphoria (Bogin et al., 1986; McKinney and Coyle, 2006; Smith and Barnes, 1983; Wiese et al., 2000). This milder form of withdrawal following acute binges of ethanol consumption is postulated by some (e.g. Smith and Barnes, 1983) to discourage further abuse of alcohol to avoid post-intoxication withdrawal or “hangover” consequences. Within this framework, individuals that show minimal aversive “hangover” symptoms might be at higher risk to develop patterns of excessive consumption. Consistent with this notion are findings that rats selectively bred for low alcohol consumption (LAD1 line) show increased brain reward thresholds after a single high dose (4 g/kg) of ethanol, whereas rats bred for high alcohol consumption (HAD1 line) do not (Chester et al., 2006).

However, one could postulate in addition that in individuals that do experience mild aversive symptoms in response to initial alcohol consumption but nonetheless engage in repeat episodic drinking, progressive increases in severity of withdrawal signs with successive episodes may contribute to escalation of alcohol intake as the individual recognizes that drinking effectively alleviates the negative emotional state experienced during abstinence. In this fashion, acute withdrawal symptoms from individual bouts of drinking may play an increasing role over time as a motivational stimulus for further drinking through a process of negative reinforcement. In addition to positive reinforcing consequences of ethanol intake, individual variability in sensitivity (or insensitivity) to acute withdrawal effects could therefore be another factor that affects propensity to repeat binge drinking and the transition from casual drinking to loss of control and ultimate development of alcoholism (Edwards, 1990; Hershon, 1977; Koob, 2006; Koob et al., 2004; Schulteis et al., 1995; Valdez and Koob, 2004).

Animal models of ethanol withdrawal encompass a spectrum of withdrawal signs that closely parallel both somatic/autonomic and affective/emotional symptoms of withdrawal noted in humans (Becker, 2000; Goldstein, 1976; Hunter et al., 1973; Majchrowicz, 1977). Within the affective domain, elevations in brain reward thresholds and anxiogenic-like behavior are commonly reported signs in rodents undergoing withdrawal from chronic ethanol exposure (Baldwin et al., 1991; File, 1994; Lal et al., 1988; Overstreet et al., 2002; Schulteis et al., 1995). It is noteworthy that these emotional signs of withdrawal are also common to other drugs of abuse, including psychostimulants such as cocaine and amphetamines (Basso et al., 1999; Harris and Aston-Jones, 1993; Leith and Barrett, 1976; Lin et al., 2000; Markou and Koob, 1991), opioids such as heroin and morphine (Schaefer and Michael, 1986; Schulteis et

al., 1994), and nicotine (Epping-Jordan et al., 1998), despite the fact that the spectrum of physiological and somatic signs of withdrawal vary widely across these different classes of abused drugs (see Koob and Le Moal, 2005). Therefore, affective dysregulation may be common to withdrawal across a wide range of abused drugs, suggesting that delineation of the substrates mediating these responses may have broad applicability to drug abuse and addiction (Koob, 2006; Schulteis et al., 1995).

Work with rodents has confirmed that affective signs of ethanol withdrawal such as brain reward deficits and anxiogenic-like behavior as measured by generalization to the discriminative stimulus effects of the anxiogenic and pro-convulsant drug pentylenetetrazole (PTZ) emerge in a time-dependent fashion during withdrawal from acute bouts of ethanol intoxication at high doses (2–4 g/kg) that achieve peak blood alcohol levels (BAL) in the range of 250–450 mg% (Gauvin et al., 1989; Gauvin et al., 1992; Schulteis and Liu, 2006). In addition, three repeat treatments at weekly intervals resulted in an extended duration of brain reward threshold elevations (Schulteis and Liu, 2006), indicating that even occasional binges of ethanol intoxication can result in a progressive increase in post-intoxication “hangover” signs.

To complement this work with brain reward deficits, we sought to optimize a simple model of the anxiogenic components of acute ethanol withdrawal that would not require extensive prior training of the animals, and exposure to other drugs (e.g. the PTZ drug discrimination model) for use in future studies of the neuroanatomical and neurochemical substrates mediating these initial neuroadaptive responses. The elevated plus maze, used extensively in our prior work on withdrawal from chronic alcohol and opioids (Baldwin et al., 1991; Rassnick et al., 1993; Schulteis et al., 1998; Valdez et al., 2002; Valdez et al., 2004; Valdez et al., 2003), has recently been shown to be sensitive to withdrawal from acute ethanol treatment at a high dose (4 g/kg) and a single test time (15 hr post-ethanol; Doremus et al., 2003). The aim of the present study was to verify the sensitivity of the relatively simple elevated plus-maze technique to acute withdrawal from ethanol, and to vary systematically several factors such as dose of ethanol, time post-injection, and acute vs. repeated intermittent treatment to achieve several goals: 1) to characterize the time course for expression of anxiogenic-like behavior; 2) to examine directly whether repeated bouts of ethanol intoxication would result in an increase in duration of withdrawal-associated anxiogenic-like behavior, as we have observed for brain reward deficits (Schulteis and Liu, 2006); and 3) to determine the minimum dose of ethanol that would elicit anxiogenic-like withdrawal signs after acute treatment, and potentiation of withdrawal after repeat treatment.

MATERIALS AND METHODS

Animals

Subjects (n = 260) were naive male Wistar rats (Harlan Labs, Indianapolis, IN, USA) weighing 270 - 300 g at the time of testing. Animals were group housed (2–3 per cage) in standard rat cages in a temperature- and humidity-controlled environment, and had free access to rat chow and water at all times. Lighting in the vivarium room was kept on 12 L: 12 D cycle, with lights on at 06:00 hr. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the VA San Diego Healthcare System, an AAALAC-accredited facility, and are in strict accordance with the “Guide for the Care and Use of Laboratory Animals” (revised 1996).

Ethanol Preparation and Injection

Ethanol (15% w/v) was prepared by diluting a 95% stock solution of ethanol with distilled water. Animals received intraperitoneal (IP) injections of a 15% ethanol solution at doses of 1.0, 2.0, or 3.0 g/kg in a volume of 0.67, 1.3, or 2.0ml/100 g body weight, respectively. As

described previously (Morse et al., 2000; Schulteis and Liu, 2006), volume instead of ethanol concentration was adjusted to vary dose to avoid potential discomfort resulting from IP injection of ethanol concentrations higher than 15%.

Elevated Plus-Maze Apparatus and Procedure

The plus-maze apparatus (Kinder Scientific, Poway CA) was constructed from black plexiglas and consisted of two opposing open arms (Length: 50 cm, Width 10.8 cm), bounded by 4-mm-high ledges on the sides and at the end of the arms to prevent slippage, and two opposing closed arms of equal length and width but with 33.5 cm high walls on all sides except the 10.8 cm-wide entrance to the center of the maze. The center of the maze was a 10.8×10.8 cm square area from which each of the four arms was connected at 90° relative to the adjacent arms. The maze floor was elevated 85 cm from the floor of the testing room. Testing was conducted in a quiet room with a white noise generator providing approximately 65 dB background noise. The testing room was illuminated only by two 25-W light bulbs in clip-on fixtures that were attached to the legs of the enclosed arms and positioned to direct light against the walls of the testing room behind each closed arm.

To begin a test session, rats were placed in the center of the maze facing towards one of the enclosed arms. Photo beam arrays embedded along the entire length of the base of each closed arm and the entry point to all arms continuously tracked position of the rat in the closed arms and center of the maze. Position in the open arms was tracked by photo beam arrays that were embedded in clear Plexiglas tubes that extended from the distal end of each closed arm and ran parallel to the open arms. Data was collected and analyzed by a computer using MotorMonitor Software (Kinder Scientific, Poway CA). Between each trial, the maze was cleaned with a damp sponge and dried with paper towels.

Experimental Design

Six to 9 days after arrival in the colony, rats were transported to a testing suite that consisted of a front “holding” room where rats could be handled, weighed, injected, and housed before and after testing, and a back “testing” room where the rats were exposed to the maze. The illumination and background noise in the holding room matched that described above for the testing room. Thirty to 60 min after transport to the holding room, each rat was gently handled for 5 min, weighed, injected with vehicle or ethanol, then returned to their home cage and kept in the room for approximately four hours to acclimate to the ambient lighting and noise conditions before being returned to the vivarium room. This procedure was repeated on each of two consecutive days. On the third day when testing took place, all rats were again moved to the holding room, weighed, injected with vehicle or ethanol, and placed back into their own home cages with food and water freely available. At 3, 6, 9, 12, 15 or 24 hr after the third and final injection, rats were allowed to freely explore the maze for 5 min. All maze testing took place between 09:00 and 16:00 hr, with injection times on the test day varying to produce the appropriate interval between ethanol injection and test. The interval between each of the three injections was held constant at approximately 24 hr. For each withdrawal interval tested, separate groups of rats received either a) vehicle for all three injections (NAIVE); b) vehicle for the first two injections and ethanol (1.0, 2.0, or 3.0 g/kg in separate cohorts) for the third injection (ACUTE); or c) ethanol (1.0, 2.0, or 3.0 g/kg) for all three injections (REPEAT). Each rat was tested only once. The same investigator performed all habituation, injection and testing procedures with a given group of rats.

For each dose of ethanol tested (1.0, 2.0 and 3.0 g/kg), initial study design included separate cohorts of rats tested under NAIVE, ACUTE, and REPEAT conditions at two successive time points, beginning with a time point where blood alcohol levels (BAL) first declined to zero at the given dose as determined in our prior work under identical experimental conditions

(Schulteis and Liu, 2006). The selection of these initial time points (3–6 hr for 1.0 g/kg, 6–9 hr for 2.0 g/kg, and 9–12 hr for 3.0 g/kg) is based upon earlier work by Gauvin and colleagues (Gauvin et al., 1989; Gauvin et al., 1992), Spear and colleagues (Doremus et al., 2003), and our group (Morse et al., 2000; Schulteis and Liu, 2006) which indicated that behavioral measures of withdrawal typically peak near the time where BAL first decline to undetectable levels.

Where effects were observed at a given dose at one or both of the initial time points selected for study, earlier and/or later time points were added as needed for all treatment conditions (NAIVE, ACUTE, REPEAT) to fully characterize the time course for each dose while minimizing animal subjects requirements by avoiding unnecessary testing at multiple ineffective time points. Based upon this strategy, only 3 and 6 hr time points were tested at 1.0 g/kg ethanol. For the 2.0 g/kg dose, 3 hr, 12 hr, and 15 hr time points were ultimately added to the analysis. For the 3.0 g/kg dose, 6, 15 and 24 hr time points were added, but a complete study was not possible at the 6 hr time point because 75% (6/8) of rats tested in the first cohort at 6 hr (both ACUTE and REPEAT) fell from the open arms of the maze during testing, presumably due to marked ataxia from high residual blood alcohol levels (> 220 mg%, see Schulteis and Liu, 2006).

Data Analysis

From the computer-recorded data, the following measures were computed for each rat: 1) time spent in the open arms as a percentage of the total time spent exploring both the open and closed arms (Percent Time); 2) number of entries into the open arms as a percentage of the total number of entries into both open and closed arms (Percent Entries); and 3) the total number of entries into the closed arms (Closed Entries). Our primary measure of interest was Percent Time in the open arms, because this measure has consistently been shown in factor analyses to have the highest loading on the factor representing the “anxiety” dimension (Cruz et al., 1994; Fernandes and File, 1996; Ohl et al., 2001; Rodgers and Dalvi, 1997; Wall and Messier, 2001), and has proven to be among the most reliable indices of anxiogenic-like effects of drug withdrawal (Baldwin et al., 1991; Doremus et al., 2003; File, 1994; Kliethermes, 2005; Rassnick et al., 1993; Schulteis et al., 1998; Valdez et al., 2002; Valdez et al., 2004; Valdez et al., 2003). Our secondary measure of anxiogenic-like behavior was Percent Entries into the open arms, which also shows a high loading on “anxiety” dimensions in factor analyses, but can occasionally yield negative results in ethanol withdrawal studies when hypoactivity is a prominent component of withdrawal (e.g. Baldwin et al., 1991). Finally, our measure of general activity was Closed Entries, a reliable and validated index of locomotor activity in the maze (e.g. Fernandes and File, 1996; File, 1994, 1995; Kliethermes, 2005).

Data for each dose of ethanol (1.0, 2.0, 3.0 g/kg) were analyzed separately due to differences in time points examined at each dose (see Experimental Design for details). All data for the primary measure of interest, Percent Time, were initially entered into two-factor ANOVAs with treatment condition (NAIVE, ACUTE, REPEAT) and time post-injection as between-subjects factors. Follow-up comparisons consisted of simple main effects of treatment condition at individual time points. All significant simple main effects were further analyzed through individual means comparisons among pairs of treatment conditions using the Newman-Keuls correction for multiple comparisons. For all simple main effects that were significant for the primary anxiogenic index of Percent Time, the identical simple main effects comparisons were made for the Percent Entries and Closed Entries measures, with significant effects again being further probed by individual means comparisons corrected by Newman-Keuls.

RESULTS

For the 1.0 g/kg dose of ethanol, inspection of Figure 1 suggests a possible reduction in Percent Time in open arms for the REPEAT condition tested at 3 hr, but overall analysis of the data detected no significant main effects or interaction of treatment and time factors for the Percent Time, Percent Entries, or Closed Entries measures (all F 's < 2.14, p 's > 0.13). Therefore, no follow-up analyses of the 1.0 g/kg data were undertaken.

As shown in Figure 2, ACUTE and REPEAT ethanol treatment at the 2.0 g/kg dose produced a time-dependent anxiogenic-like effect as measured by the primary measure of Percent Time; this was confirmed by a significant interaction of treatment condition (NAIVE, ACUTE, REPEAT) and time (3, 6, 9, 12, 15 hr post-injection) ($F[8, 129] = 2.50$, $p < 0.02$). Simple main effects of treatment at each time point indicated this interaction was due to treatment-specific effects at 6 and 9 hr post-ethanol ($F[2, 28] = 4.97$, $p < 0.02$ and $F[2, 28] = 5.49$, $p < 0.01$, respectively), an effect that approached significance at 12 hr ($F[2, 23] = 2.63$, $p < .10$), and no significant effects at 3 or 15 hr post-injection (F 's < 1.50, p 's > 0.24). Individual means comparisons at 6 and 9 hr post-injection revealed that ACUTE ethanol significantly reduced exploration of the open arms relative to NAIVE controls only at 6 hr post-ethanol, whereas REPEAT ethanol extended the time course of effect to include 9 hr as well (a marginal effect in the REPEAT group at 12 hr appeared to account for the trend toward significant main effect of treatment at this time point).

For the measure of Percent Entries, an inspection of Figure 2 suggests reduced sensitivity to withdrawal-induced effects, and analysis of simple main effects at 6 and 9 hr post-injection confirms this impression. At 9 hr post-ethanol, there was a significant reduction in Percent Entries with REPEAT but not ACUTE ethanol treatment (simple main effect: $F[2, 28] = 3.81$, $p < 0.05$; NAIVE vs. REPEAT: $p < 0.05$ after Newman-Keuls correction; NAIVE vs. ACUTE not significant), mirroring the pattern of results obtained for the Percent Time measure. However, at 6 hr post-ethanol where both ACUTE and REPEAT treatment reduced Percent Time in the open arms, ACUTE treatment with 2.0 g/kg of ethanol appeared to reduce Percent Entries into the open arms (see Figure 2), but REPEAT treatment was not different from NAIVE controls, and the overall simple main effect of treatment condition approached but did not achieve two-tailed significance at this time point ($F[2, 28] = 2.61$, $p < 0.10$). The lack of effect of REPEAT 2.0 g/kg on the Percent Entries measure at 6 hr post-injection was accompanied by a significant reduction in the total number of Closed Entries, confirmed by a significant simple main effect of treatment ($F[2, 28] = 13.78$, $p < 0.0001$) and post-hoc Newman-Keuls comparison of NAIVE and REPEAT treatment groups ($p < 0.05$; NAIVE vs. ACUTE not significant). By 9 hr post-injection there was no longer a significant change in Closed Entries ($F[2, 28] = 0.26$, $p > 0.75$).

Analysis of Percent Time data for the 3.0 g/kg dose revealed a treatment $\times$ time interaction that did not quite achieve significance ($F[6, 92] = 1.95$, $p < 0.08$), perhaps due to the broader range of time points where significant effects were observed under both ACUTE and REPEAT treatment conditions with 3.0 g/kg (see Figure 3) in comparison to the transient effects at 2.0 g/kg (Figure 2). However, the overall ANOVA did reveal significant main effects of treatment ($F[2, 92] = 12.66$, $p < 0.0001$) and time ($F[3, 92] = 3.62$, $p < 0.02$). Further analysis of the simple main effects revealed significant treatment-specific effects at 9 hr ($F[2, 28] = 10.59$, $p < 0.001$), 12 hr ($F[2, 21] = 4.71$, $p < 0.02$), and 15 hr post-ethanol ($F[2, 22] = 5.24$, $p < 0.02$), but not at 24 hr ($F[2, 21] = 0.04$, $p > 0.95$). Follow-up individual means comparisons of ACUTE and REPEAT ethanol groups to NAIVE controls at 9, 12, and 15 hr revealed significant reductions in percent open arm time at 9 and 12 hr after ACUTE treatment, and 9, 12, and 15 hr after REPEAT treatment (all $p < 0.05$ after Newman-Keuls correction).

Turning to the Percent Entries and Closed Entries measures for the 3.0 g/kg dose of ethanol, Figure 3 again illustrates a reduced sensitivity of the Percent Entries measure as compared to Percent Time. At 9 hr post-injection, there was a significant main effect of treatment on Percent Entries ($F[2,28] = 6.53, p < 0.005$), and post-hoc comparisons indicated significant reductions in Percent Entries relative to NAIVE controls under both ACUTE and REPEAT treatment conditions. A significant main effect of treatment on Closed Entries was also observed at this time point ($F[2,28] = 5.94, p < 0.001$), with follow-up analysis indicating that only the REPEAT group was significantly different from NAIVE controls. By 12 hr post-injection, there was no significant main effect of treatment on Percent Entries ($F[2,21] = 0.83, p > 0.45$), but there was a main effect of treatment on Closed Entries ($F[2,21] = 5.59, p < 0.02$) attributable to a significant reduction in activity under REPEAT treatment conditions, but not ACUTE, as compared to NAIVE controls (Newman-Keuls post-hoc). This same pattern of significant activity effect ($F[2,22] = 7.97, p < 0.005$) and no significant effect on Percent Entries ($F[2,22] = 1.16, p > 0.30$) was observed at 15 hr post-injection; once again, the significant effect on Closed Entries was due to a reduction in this measure with REPEAT treatment, but not ACUTE (as compared to NAIVE with Newman-Keuls correction).

DISCUSSION

The current study confirmed an earlier report of anxiogenic-like behavior in the elevated plus maze during withdrawal from single bouts of ethanol intoxication (Doremus et al., 2003), and extended this report by providing a parametric analysis of the dose- and time-dependent expression of this negative emotional withdrawal state. In the present study, a low dose of ethanol (1.0 g/kg), which produces peak BAL of 120 mg% in the Wistar strain of rats used herein, was ineffective at eliciting anxiogenic-like behavior from 0–3 hr after BAL reached undetectable levels (3–6 hr post-injection; see Schulteis and Liu, 2006, Figure 1). However, a dose of 2.0 g/kg induced a transient but marked reduction in exploration of the open arms of the elevated plus maze at 6 hr post-injection, a time when BAL first declines to undetectable levels at this dose (see Schulteis and Liu, 2006). Treatment with 3.0 g/kg ethanol, which produces peak BAL of 410 mg% in this rat strain, resulted in a significant anxiogenic-like effect at 9–12 hr post-injection, again beginning at a time when BAL neared zero (Schulteis et al., 2006). Taken together with the report of Doremus et al. (2003) that withdrawal from acute injection of 4.0 g/kg ethanol elicits anxiogenic-like behavior at 18 hr (3 hr after BAL were undetectable), the present findings suggest that peak anxiogenic-like behavior as measured in the elevated plus maze during acute withdrawal “hangover” varies in a dose-dependent fashion to occur at or soon after the complete metabolism of ethanol; earlier time course analyses using the PTZ drug discrimination model (Gauvin et al., 1989, 1992) are also consistent with this notion.

In addition to characterizing the time course and dose-effect function for acute ethanol “hangover”-induced anxiogenic-like behavior in the elevated plus maze, the present study also demonstrated that as few as three daily bouts of intoxication and withdrawal result in a significant increase in the duration of anxiogenic-like behavior accompanying acute ethanol “hangover”. Whereas repeat treatment with 1.0 g/kg produced no significant reduction in Percent Time in the open arms of the maze, repeat treatment with 2.0 g/kg produced an extended duration of anxiogenic-like behavior from 6–12 hr post-injection, corresponding closely to the time course of peak brain reward threshold elevations reported in our earlier work (Schulteis and Liu, 2006). Moreover, withdrawal from an acute treatment with the highest dose of ethanol tested (3.0 g/kg) produced significant anxiogenic-like behavior from 9–12 hr post-injection, but three daily injections of this same dose extended the time course of effect to at least 15 hr post-treatment (the effect having dissipated by 24 hr). Taken together with our prior findings on elevated brain reward thresholds (Schulteis and Liu, 2006), the present findings support the notion of a rapid increase in duration of withdrawal-associated negative affective states after

just a few bouts of ethanol intoxication and withdrawal. This is consistent with reports of rapid progression of affective components of withdrawal from 1–4 acute intermittent treatments with morphine (Liu and Schulteis, 2004; Schulteis et al., 2004; Schulteis and Zhang, 2006; Zhang and Schulteis, submitted), and suggests that emotional or affective circuitry in the brain may undergo rapid neuroadaptation during initial use of drugs of abuse such as alcohol and opioids.

Rats in the REPEAT treatment groups of the current study experienced 10–18 hr of abstinence (as defined by undetectable BAL) after each of the three bouts of intoxication, therefore one must consider the possibility that repeated daily abstinence may have induced withdrawal “kindling”. The “kindling” hypothesis of alcohol withdrawal, proposed by Ballenger and Post in 1978, states that severity of withdrawal is directly potentiated by repeated episodes of abstinence interspersed between heavy episodes of drinking. Both retrospective analyses of patient history in alcohol treatment programs (Ballenger and Post, 1978; Booth and Blow, 1993; Brown et al., 1988; Schuckit et al., 1995) and work with animal models support the ability of repeated bouts of withdrawal to “kindle” withdrawal severity as measured by seizure susceptibility and other physiological and somatic markers (Baker and Cannon, 1979; Becker et al., 1997; Ulrichsen et al., 1992; Veatch and Gonzalez, 1996). Moreover, several studies indicate that repeated intoxication and withdrawal from chronic (Roberts et al., 2000; Schulteis et al., 1996) or more acute (Becker and Lopez, 2004) regimens of ethanol exposure potentiated subsequent ethanol consumption in limited access bouts of bottle-drinking or operant self-administration, suggesting motivational relevance of the repeated intoxication and/or withdrawal experience.

Of direct relevance to the withdrawal sign under investigation in the present study, recent work indicates that anxiogenic-like behavior as measured in the social interaction test is subject to potentiation by repeated bouts of ethanol withdrawal (Breese et al., 2005; Overstreet et al., 2002; Overstreet et al., 2004). However, in contrast to the transient bouts of intoxication produced by daily intraperitoneal injections of ethanol in the present study, the work by Overstreet, Breese and colleagues utilized more protracted exposure and withdrawal conditions, specifically repeated bouts of five days continuous access to ethanol liquid diet interspersed with two days forced abstinence. Our findings of potentiated anxiogenic-like behavior and brain reward deficits with repeated acute bouts of ethanol intoxication and withdrawal are consistent with this earlier work, and furthermore suggest that even acute “binge” patterns of intoxication followed by daily (current study) or more extended (weekly; Schulteis and Liu, 2006) periods of abstinence can lead to potentiation of negative emotional states.

Characterization of anxiogenic-like behavior during ethanol withdrawal as measured in the elevated plus maze must address hypoactivity as an additional behavioral withdrawal sign of ethanol withdrawal, commonly observed after chronic ethanol treatment (Baldwin et al., 1991; File, 1994, 1995; Kliethermes, 2005), or acute withdrawal from a very high dose of ethanol (4.0 g/kg; Doremus et al., 2003). Considerable attention has been given to the possible confound of drug effects on traditional indices of anxiety in the elevated plus maze by nonspecific effects on activity or exploration (File, 1995; Kliethermes, 2005; Rodgers and Dalvi, 1997; Wall and Messier, 2001; Weiss et al., 1998). In the case of ethanol withdrawal, however, there are several distinct types of empirical evidence that withdrawal-induced anxiogenic-like behavior is dissociable from withdrawal-induced hypoactivity.

First, in the present study hypoactivity as measured by Closed Entries emerged only upon repeat intoxication and withdrawal at the doses of ethanol employed in the present study, whereas anxiogenic-like behavior as inferred from Percent Time and Percent Entries measures was reduced relative to NAIVE control conditions after both ACUTE and REPEAT ethanol intoxication. Thus, withdrawal from acute ethanol intoxication can result in time-dependent

expression of anxiogenic-like behavior that is dissociable from withdrawal-induced hypoactivity at lower doses of ethanol (2.0 – 3.0 g/kg). In contrast, under conditions of REPEAT ethanol treatment, we did observe ethanol-induced hypoactivity as measured by reductions in total closed arm entries at most time intervals where anxiogenic-like effects were present, and it appears as though the Percent Entries (but not Percent Time) measure may have been potentially confounded by the reduction in total closed arm entries (see for example REPEAT condition in Figure 2 at 6 hr, Figure 3 at 12–15 hr). Reduced sensitivity of the Percent Entries as compared to Percent Time measure to detect significant anxiogenic-like effects was also noted in earlier work under conditions where withdrawal from chronic ethanol was accompanied by significant effects on general activity as measured by total arm entries (e.g Baldwin et al., 1991).

Second, Doremus et al. demonstrated at a developmental level that adolescent rats show acute withdrawal-induced hypoactivity but not anxiogenic-like behavior in the elevated plus maze, whereas adult rats exhibited both behaviors. Third, File (1994) reported that chronic noise stress presented during the induction of ethanol dependence could abolish the anxiogenic-like consequences of withdrawal in both elevated plus maze and social interaction tests, but that withdrawal-induced hypoactivity was unaffected by noise stress exposure. Finally, Koob and colleagues demonstrated that a corticotropin-releasing factor (CRF) antagonist administered into the cerebral ventricles of rats could similarly reverse anxiogenic-like effects of withdrawal from either chronic ethanol exposure or a single bout of intoxication with a 3 g/kg dose as measured by percent time in the open arms of an elevated plus maze (Baldwin et al., 1991; Morse and Koob, unpublished observations). However, this same CRF antagonist did not reverse withdrawal hypoactivity as measured by total entries into open and closed arms (Baldwin et al., 1991). This dissociation may exist at the neuroanatomical level as well, given that the anxiogenic-like effects of corticotropin-releasing factor (CRF) injected directly into the bed nucleus of the stria terminalis are not accompanied by significant changes in activity in the elevated plus maze (Sahuque et al., 2006). Therefore, with specific reference to the study of ethanol withdrawal, it appears that the Percent Time measure in the elevated plus maze reliably detects anxiogenic-like effects during abstinence from acute, repeat intermittent, or chronic ethanol exposure that are dissociable from withdrawal-induced hypoactivity, but that the Percent Entries measure may be a less reliable index when activity effects are present (Baldwin et al., 1991, present study).

In summary, negative emotional states accompanying withdrawal from acute ethanol intoxication at a moderate dose (2.0 g/kg) include anxiogenic-like behavior (present study) and elevated brain reward thresholds (Schulteis et al., 2006), with both signs showing peak effects at 6 hr following a single bout of intoxication, a time point where BAL first declines to undetectable. Repeat treatment with this same dose of ethanol results in an increased duration of effect, with peak effects noted from 6–12 hr post-ethanol in both the elevated plus maze and brain stimulation reward measures. Notably, both of these negative emotional signs of acute ethanol withdrawal “hangover” are clearly demonstrable at a dose of ethanol (2.0 g/kg) that produces peak average BAL of 270 ± 25 mg% in our strain of Wistar rats (see Schulteis et al., 2006); this BAL falls into a range that human “binge” drinkers might typically experience (200–300 mg%). As mentioned earlier, substantial evidence supports the notion that abstinence-associated anxiety, dysphoria, or depressed mood play a significant role in the maintenance of continued drug craving and relapse in addicted individuals (Bokstrom et al., 1989; Bokstrom et al., 1991; Roelofs, 1985; Roelofs and Dikkenberg, 1987). Work with the acute ethanol withdrawal “hangover” model now indicates that these same negative emotional states are invoked at the earliest stages of drug use, where they may serve as an additional factor beyond the positive reinforcing effects of ethanol consumption in the transition from casual use to loss of control and compulsive use that characterize addiction (Koob, 1996; Liu and Schulteis, 2004; Schulteis and Liu, 2006).

Within this context, it is noteworthy that brain reward deficits and anxiogenic-like behavior during withdrawal from acute ethanol (present study, Schulteis and Liu, 2006) closely parallel negative affective consequences of withdrawal from acute opioid treatment (Liu and Schulteis, 2004; Schulteis and Zhang, 2006, submitted). Throughout these studies it has been clearly demonstrated that withdrawal severity is potentiated with repeated intermittent daily or weekly exposure to opioids or ethanol, suggesting rapid induction and progression of neuroadaptation within brain emotional and reward circuitry. Animal models of these negative affective consequences of withdrawal from acute and repeated intermittent ethanol intoxication such as those developed herein and elsewhere (Schulteis and Liu, 2006) should prove useful in delineating the specific neuroanatomical and neurochemical substrates within brain reward, emotional, and stress circuitry (e.g. CRF systems in the CNS) that are critical to initial neuroadaptation to ethanol.

Acknowledgements

This work was supported by NIAAA grants AA12800 (GS), AA08459 (GFK), and NIAAA center grant AA06420 (GFK). Andrew Morse was supported by NIAAA training grant T32-AA07456.

This is publication 18886 from the Scripps Research Institute. Portions of these data were presented in initial form as a poster at the 2006 annual meeting of the Society for Neuroscience (Zhang et al., 2006).

References

- Annis HM, Sklar SM, Moser AE. Gender in relation to relapse crisis situations, coping, and outcome among treated alcoholics. *Addict Behav* 1998;23(1):127–31. [PubMed: 9468752]
- Baker TB, Cannon DS. Potentiation of ethanol withdrawal by prior dependence. *Psychopharmacology (Berl)* 1979;60(2):105–10. [PubMed: 106420]
- Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT. CRF antagonist reverses the “anxiogenic” response to ethanol withdrawal in the rat. *Psychopharmacology (Berl)* 1991;103(2):227–32. [PubMed: 2027923]
- Ballenger JC, Post RM. Kindling as a model for alcohol withdrawal syndromes. *Br J Psychiatry* 1978;133:1–14. [PubMed: 352467]
- Basso AM, Spina M, Rivier J, Vale W, Koob GF. Corticotropin-releasing factor antagonist attenuates the “anxiogenic-like” effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats. *Psychopharmacology (Berl)* 1999;145(1):21–30. [PubMed: 10445369]
- Becker HC. Animal models of alcohol withdrawal. *Alcohol Res Health* 2000;24(2):105–13. [PubMed: 11199277]
- Becker HC, Diaz-Granados JL, Weathersby RT. Repeated ethanol withdrawal experience increases the severity and duration of subsequent withdrawal seizures in mice. *Alcohol* 1997;14(4):319–26. [PubMed: 9209546]
- Becker HC, Lopez MF. Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. *Alcohol Clin Exp Res* 2004;28(12):1829–38. [PubMed: 15608599]
- Bogin RM, Nostrant TT, Young MJ. Propranolol for the treatment of the alcoholic hangover. *Am J Drug Alcohol Abuse* 1986;12(3):279–84. [PubMed: 3503570]
- Bokstrom K, Balldin J, Langstrom G. Alcohol withdrawal and mood. *Acta Psychiatr Scand* 1989;80(5):505–13. [PubMed: 2596351]
- Bokstrom K, Balldin J, Langstrom G. Individual mood profiles in alcohol withdrawal. *Alcohol Clin Exp Res* 1991;15(3):508–13. [PubMed: 1877737]
- Booth BM, Blow FC. The kindling hypothesis: further evidence from a U.S. national study of alcoholic men. *Alcohol* 1993;28(5):593–8. [PubMed: 8274184]
- Breese GR, Overstreet DH, Knapp DJ. Conceptual framework for the etiology of alcoholism: a “kindling”/stress hypothesis. *Psychopharmacology (Berl)* 2005;178(4):367–80. [PubMed: 15765253]

- Brown ME, Anton RF, Malcolm R, Ballenger JC. Alcohol detoxification and withdrawal seizures: clinical support for a kindling hypothesis. *Biol Psychiatry* 1988;23(5):507–14. [PubMed: 3345323]
- Chester JA, Rausch EJ, June HL, Froehlich JC. Decreased reward during acute alcohol withdrawal in rats selectively bred for low alcohol drinking. *Alcohol Clin Exp Res* 2006;38(3):165–72.
- Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav* 1994;49(1):171–6. [PubMed: 7816869]
- De Soto CB, O'Donnell WE, De Soto JL. Long-term recovery in alcoholics. *Alcohol Clin Exp Res* 1989;13(5):693–7. [PubMed: 2688470]
- Doremus TL, Brunell SC, Varlinskaya EI, Spear LP. Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. *Pharmacol Biochem Behav* 2003;75(2):411–8. [PubMed: 12873633]
- Edwards G. Withdrawal symptoms and alcohol dependence: fruitful mysteries. *Br J Addict* 1990;85(4):447–61. [PubMed: 2189511]
- Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* 1998;393(6680):76–9. [PubMed: 9590692]
- Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacol Biochem Behav* 1996;54(1):31–40. [PubMed: 8728536]
- File SE. Chronic exposure to noise modifies the anxiogenic response, but not the hypoactivity, detected on withdrawal from chronic ethanol treatment. *Psychopharmacology (Berl)* 1994;116(3):369–72. [PubMed: 7892429]
- File, SE. Animal models of different anxiety states. In: Biggio, G.; Sanna, E.; Costa, E., editors. *GABA_A Receptors and Anxiety: From Neurobiology to Treatment*. Raven Press; New York: 1995. p. 93-113.
- Gauvin DV, Harland RD, Criado JR, Michaelis RC, Holloway FA. The discriminative stimulus properties of ethanol and acute ethanol withdrawal states in rats. *Drug Alcohol Depend* 1989;24(2):103–13. [PubMed: 2791886]
- Gauvin DV, Youngblood BD, Holloway FA. The discriminative stimulus properties of acute ethanol withdrawal (hangover) in rats. *Alcohol Clin Exp Res* 1992;16(2):336–41. [PubMed: 1590555]
- Goldstein DB. Pharmacological aspects of physical dependence on ethanol. *Life Sci* 1976;18(6):553–61. [PubMed: 4686]
- Harris GC, Aston-Jones G. Beta-adrenergic antagonists attenuate withdrawal anxiety in cocaine- and morphine-dependent rats. *Psychopharmacology (Berl)* 1993;113(1):131–6. [PubMed: 7862819]
- Hershon HI. Alcohol withdrawal symptoms and drinking behavior. *J Stud Alcohol* 1977;38(5):953–71. [PubMed: 881849]
- Hunter BE, Boast CA, Walker DW, Zornetzer SF. Alcohol withdrawal syndrome in rats: neural and behavioral correlates. *Pharmacol Biochem Behav* 1973;1(6):719–25. [PubMed: 4594650]
- Kliethermes CL. Anxiety-like behaviors following chronic ethanol exposure. *Neurosci Biobehav Rev* 2005;28(8):837–50. [PubMed: 15642625]
- Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. *Addiction* 2006;101(Suppl 1):23–30. [PubMed: 16930158]
- Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, O'Dell LE, Parsons LH, Sanna PP. Neurobiological mechanisms in the transition from drug use to drug dependence. *Neurosci Biobehav Rev* 2004;27(8):739–49. [PubMed: 15019424]
- Koob, GF.; Le Moal, M. *Neurobiology of Addiction*. Academic Press; San Diego: 2005.
- Lal H, Harris CM, Benjamin D, Springfield AC, Bhadra S, Emmett-Oglesby MW. Characterization of a pentylenetetrazol-like interoceptive stimulus produced by ethanol withdrawal. *J Pharmacol Exp Ther* 1988;247(2):508–18. [PubMed: 3183950]
- Leith NJ, Barrett RJ. Amphetamine and the reward system: evidence for tolerance and post-drug depression. *Psychopharmacologia* 1976;46(1):19–25. [PubMed: 1257363]
- Lin D, Koob GF, Markou A. Time-dependent alterations in ICSS thresholds associated with repeated amphetamine administrations. *Pharmacol Biochem Behav* 2000;65(3):407–17. [PubMed: 10683480]
- Liu J, Schulteis G. Brain reward deficits accompany naloxone-precipitated withdrawal from acute opioid dependence. *Pharmacol Biochem Behav* 2004;79(1):101–8. [PubMed: 15388289]

- Majchrowicz E. Comparison of ethanol withdrawal syndrome in humans and rats. *Adv Exp Med Biol* 1977;85B:15–23. [PubMed: 596277]
- Markou A, Koob GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology* 1991;4(1):17–26. [PubMed: 2003866]
- McKinney A, Coyle K. Alcohol hangover effects on measures of affect the morning after a normal night's drinking. *Alcohol* 2006;41(1):54–60. [PubMed: 16260448]
- Mendelson JH, Mello NK. Medical progress. Biologic concomitants of alcoholism. *N Engl J Med* 1979;301(17):912–21. [PubMed: 481538]
- Metten, P.; Crabbe, JC. Dependence and withdrawal. In: Deitrich, RA.; Erwin, VG., editors. *Pharmacological Effects of Ethanol on the Nervous System*. CRC Press; Boca Raton, FL: 1996. p. 269–291.
- Miller WR, Harris RJ. A simple scale of Gorski's warning signs for relapse. *J Stud Alcohol* 2000;61(5):759–65. [PubMed: 11022817]
- Morse AC, Schulteis G, Holloway FA, Koob GF. Conditioned place aversion to the "hangover" phase of acute ethanol administration in the rat. *Alcohol* 2000;22(1):19–24. [PubMed: 11109024]
- Ohl F, Toschi N, Wigger A, Henniger MS, Landgraf R. Dimensions of emotionality in a rat model of innate anxiety. *Behav Neurosci* 2001;115(2):429–36. [PubMed: 11345967]
- Overstreet DH, Knapp DJ, Breese GR. Accentuated decrease in social interaction in rats subjected to repeated ethanol withdrawals. *Alcohol Clin Exp Res* 2002;26(8):1259–68. [PubMed: 12198403]
- Overstreet DH, Knapp DJ, Breese GR. Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors. *Pharmacol Biochem Behav* 2004;77(2):405–13. [PubMed: 14751471]
- Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* 1993;605(1):25–32. [PubMed: 8467387]
- Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacology* 2000;22(6):581–94. [PubMed: 10788758]
- Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev* 1997;21(6):801–10. [PubMed: 9415905]
- Roelofs SM. Hyperventilation, anxiety, craving for alcohol: a subacute alcohol withdrawal syndrome. *Alcohol* 1985;2(3):501–5. [PubMed: 4026971]
- Roelofs SM, Dikkenberg GM. Hyperventilation and anxiety: alcohol withdrawal symptoms decreasing with prolonged abstinence. *Alcohol* 1987;4(3):215–20. [PubMed: 3109449]
- Sahuque LL, Kullberg EF, McGeehan AJ, Kinder JR, Hicks MP, Blanton MG, Janak PH, Olive MF. Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. *Psychopharmacology (Berl)* 2006;186(1):122–32. [PubMed: 16568282]
- Schaefer GJ, Michael RP. Changes in response rates and reinforcement thresholds for intracranial self-stimulation during morphine withdrawal. *Pharmacol Biochem Behav* 1986;25(6):1263–9. [PubMed: 3809229]
- Schuckit, MA. *Drug and Alcohol Abuse: A Clinical Guide to Diagnosis and Treatment*. 4. Plenum Press; New York: 1995.
- Schuckit MA, Tipp JE, Reich T, Hesselbrock VM, Bucholz KK. The histories of withdrawal convulsions and delirium tremens in 1648 alcohol dependent subjects. *Addiction* 1995;90(10):1335–47. [PubMed: 8616462]
- Schulteis G, Hyytia P, Heinrichs SC, Koob GF. Effects of chronic ethanol exposure on oral self-administration of ethanol or saccharin by Wistar rats. *Alcohol Clin Exp Res* 1996;20(1):164–71. [PubMed: 8651448]
- Schulteis G, Liu J. Brain reward deficits accompany withdrawal (hangover) from acute ethanol in rats. *Alcohol* 2006;39(1):21–8. [PubMed: 16938626]
- Schulteis G, Markou A, Cole M, Koob GF. Decreased brain reward produced by ethanol withdrawal. *Proc Natl Acad Sci U S A* 1995;92(13):5880–4. [PubMed: 7597046]

- Schulteis G, Markou A, Gold LH, Stinus L, Koob GF. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. *J Pharmacol Exp Ther* 1994;271(3): 1391–8. [PubMed: 7996451]
- Schulteis G, Yackey M, Risbrough V, Koob GF. Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus-maze. *Pharmacol Biochem Behav* 1998;60(3): 727–31. [PubMed: 9678657]
- Schulteis, G.; Zhang, Z. Increased anxiety-like behavior during naloxone-precipitated withdrawal from acute opioid dependence. Presented at the 2006 meeting of the College on Problems of Drug Dependence (CPDD); June 17–22, 2006; Scottsdale, AZ. 2006.
- Smith CM, Barnes GM. Signs and symptoms of hangover: prevalence and relationship to alcohol use in a general adult population. *Drug Alcohol Depend* 1983;11(3–4):249–69. [PubMed: 6617465]
- Ulrichsen J, Clemmesen L, Hemmingsen R. Convulsive behaviour during alcohol dependence: discrimination between the role of intoxication and withdrawal. *Psychopharmacology (Berl)* 1992;107(1):97–102. [PubMed: 1589567]
- Valdez GR, Koob GF. Allostasis and dysregulation of corticotropin-releasing factor and neuropeptide Y systems: implications for the development of alcoholism. *Pharmacol Biochem Behav* 2004;79(4): 671–89. [PubMed: 15582675]
- Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, Koob GF. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. *Alcohol Clin Exp Res* 2002;26(10):1494–501. [PubMed: 12394282]
- Valdez GR, Sabino V, Koob GF. Increased anxiety-like behavior and ethanol self-administration in dependent rats: reversal via corticotropin-releasing factor-2 receptor activation. *Alcohol Clin Exp Res* 2004;28(6):865–72. [PubMed: 15201629]
- Valdez GR, Zorrilla EP, Roberts AJ, Koob GF. Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. *Alcohol* 2003;29(2):55–60. [PubMed: 12782246]
- Veatch LM, Gonzalez LP. Repeated ethanol withdrawal produces site-dependent increases in EEG spiking. *Alcohol Clin Exp Res* 1996;20(2):262–7. [PubMed: 8730216]
- Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. *Neurosci Biobehav Rev* 2001;25(3):275–86. [PubMed: 11378181]
- Weiss SM, Wadsworth G, Fletcher A, Dourish CT. Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. *Neurosci Biobehav Rev* 1998;23(2):265–71. [PubMed: 9884119]
- Wiese JG, Shlipak MG, Browner WS. The alcohol hangover. *Ann Intern Med* 2000;132(11):897–902. [PubMed: 10836917]
- Willinger U, Lenzinger E, Hornik K, Fischer G, Schonbeck G, Aschauer HN, Meszaros K. Anxiety as a predictor of relapse in detoxified alcohol-dependent patients. *Alcohol Alcohol* 2002;37(6):609–12. [PubMed: 12414556]
- Zhang, Z.; Schulteis, G.; Koob, GF. Anxiogenic-like behavior in the elevated plus-maze during withdrawal from acute ethanol: Potentiation with repeated intoxication and withdrawal experience. Presented at the 2006 annual meeting of the Society for Neuroscience; October 14–18, 2006; Atlanta, GA. 2006.
- Zhang Z, Schulteis G. Withdrawal from acute morphine dependence is accompanied by increased anxiety-like behavior in the elevated plus maze. submitted

Fig. 1.

Withdrawal from a single bout of acute ethanol intoxication at 1.0 g/kg (ACUTE) does not alter behavior of rats in the elevated plus maze as compared to vehicle-treated controls (NAIVE). Repeat treatment with this dose for 2 additional days (REPEAT) resulted in a modest but non-significant reduction in percent time spent on the open arms of the maze, and no alterations in other measured parameters. Animals were tested at 3 and 6 hr post-injection, because BAL induced by this dose of ethanol declines to undetectable by 3 hr in the Wistar strain of rats used herein and in our earlier work (Schulteis and Liu, 2006). Data represent mean $\pm$ SEM. N = 8–10 per group.

Fig. 2.

Withdrawal from a single bout of acute ethanol intoxication at 2.0 g/kg (ACUTE) elicited a transient but significant anxiogenic-like effect as measured by reduction in exploration of the open arms of the elevated plus maze at 6 hr post-injection, a time when BAL first decline to undetectable at this dose in Wistar rats (Schulteis and Liu, 2006). ACUTE treatment with this dose did not affect general activity as measured by entries into the closed arms at any time point examined. Repeat treatment with this dose for 2 additional days (REPEAT) resulted in an increased duration of anxiogenic-like effect, now significant from 6–12 hr post-injection. This effect was reliably detected with percent time in the open arms (upper panel), but was less evident with percent entries into the open arms as the dependent variable (middle panel). A

significant reduction in general activity was also seen at 6 hr after the final ethanol injection in the REPEAT treatment group. Data represent mean $\pm$ SEM. N = 8–11 per group. * $p < 0.05$ as compared to vehicle-treated controls (NAIVE).

Fig. 3.

Withdrawal from a single bout of acute ethanol intoxication at 3.0 g/kg (ACUTE) elicited a significant anxiogenic-like response as measured by reduction in exploration of the open arms of the elevated plus maze. This effect was significant from 9–12 hr post-injection, just after BAL first decline to undetectable at this dose in Wistar rats (Schulteis and Liu, 2006). ACUTE treatment with this dose did not affect general activity as measured by entries into the closed arms at any time point examined. Repeat treatment with this dose for 2 additional days (REPEAT) resulted in an increased duration of anxiogenic-like effect, now significant up to 15 hr post-injection. This extended duration of effect was reliably detected with percent time in the open arms (upper panel), but was less evident with percent entries into the open arms as

the dependent variable (middle panel), possible due to confounding of the latter measure by significant reductions in entries into the closed arms (lower panel). Data represent mean $\pm$ SEM %. N = 8–11 per group. * $p < 0.05$ as compared to vehicle-treated controls (NAIVE).