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Restraint stress and exogenous corticosterone differentially alter sensitivity to the sedative-hypnotic effects of ethanol in inbred long-sleep and inbred short-sleep mice

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Abstract

Decreased sensitivity to ethanol is a genetically mediated trait implicated in susceptibility to developing alcoholism. Here, we explore genotype by environment differences in ethanol sensitivity. The relationship between acute- and repeated-restraint stress, corticosterone (CORT) levels, and sensitivity to sedative-hypnotic properties of ethanol was explored using inbred long-sleep (ILS) and inbred short-sleep (ISS) mice. In ILS mice, acute restraint decreased ethanol sensitivity at a 4.1 g/kg dose, as measured by a decrease in the duration of loss of the righting reflex (LORE) and an increase in blood ethanol concentration at regain of the righting response (BECRR). Repeated restraint also decreased LORE duration, but had no effect on BECRR. In the ISS mice, there was no effect of acute restraint on either LORE duration or BECRR. However, repeated restraint increased ethanol sensitivity at a 4.1 g/kg dose; with an increase in LORE duration, but a decrease in BECRR. Differences in hypothalamic-pituitary-adrenal (HPA) axis responsiveness to restraint stress (as measured by plasma CORT) were also examined between genotypes. ILS mice displayed habituation to repeated restraint, whereas ISS mice did not. Lastly, the effect of enhanced CORT levels independent of psychological stress was examined for its effects on the sedative-hypnotic effects of ethanol. There were no effects of CORT pretreatment on LORE duration or BECRR in ILS mice compared to saline- or noninjected littermates. In contrast, ISS mice injected with CORT showed a decreased duration of LORE, but no effects on BECRR. These findings suggest that in addition to genetic susceptibility, environmental factors (e.g., restraint stress, exogenous CORT administration) also influence sensitivity to the sedative effects of ethanol through alteration of central nervous system sensitivity and pharmacokinetic parameters, and do so in a genotype-dependent manner.

Keywords

Corticosterone; Ethanol; Ethanol sensitivity; Inbred long-sleep mice; Inbred short-sleep mice; Loss of righting reflex; Stress; Restraint stress; BEC

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Introduction

Low initial sensitivity to the intoxicating effects of ethanol has been implicated in the development of alcoholism in humans (Schuckit, 1994a, 1994b; Schuckit & Smith, 1996) and has been shown to be genetically mediated (Baldwin et al., 1991; Crabbe, 1983; Crabbe et al., 2006; Deitrich, 1990, 1993; Heath & Martin, 1992; Kakihana et al., 1968; Li, 2000; Madden et al., 1995; Moore et al., 1998). Schuckit (1980, 1984) demonstrated that sons of alcoholic fathers, who are at an elevated risk for alcoholism, were less sensitive to ethanol than sons of nonalcoholic fathers. Following alcohol administration, sons of alcoholics reported a significantly lower level of intoxication, and displayed lower amounts of whole-body sway (Schuckit, 1985) than matched controls who were sons of nonalcoholics. In 10- and 20-year follow-up studies, Schuckit (1994a, 2004) has shown that lack of sensitivity to alcohol intoxication is a predictor of future alcohol use disorders. Presumably, a low level of response to alcohol leads to increased risk for alcoholism via increased consumption. Although the exact mechanism remains unclear, it is believed that individuals who do not feel the effects of alcohol as strongly may drink more to obtain the same effects that others experience at a lower blood alcohol level.

In addition to genetic differences, sensitivity to ethanol may also be modulated by environmental factors such as stress. One mechanism for stress-induced alterations in ethanol sensitivity may be that stress-stimulated glucocorticoid secretion alters brain neurochemistry that modifies the pharmacodynamic actions of ethanol. In fact, both stress and ethanol affect common neurotransmitter systems, including γ -aminobutyric acid (GABA), *N*-methyl-D-aspartate (NMDA), dopamine (DA), and serotonin (5HT) receptor sites (Brady & Sonne, 1999; Grant, 1994; Swiergiel et al., 2008; Wise & Bozarth, 1987). Despite the fact that both stress and ethanol act on shared neural pathways, the data on the behavioral response to ethanol in stressed mice have been mixed. Sze (1993) found that an acute dose of corticosterone (CORT) administered 15 min prior to ethanol injection significantly shortened the duration of loss of the righting reflex (LORE) in male mice by 55%. In addition, others have reported that isolate housing (a mild chronic stressor known to elevate basal CORT levels) attenuated the duration of LORE in both long-sleep (LS) and short-sleep (SS) male mice (Jones et al., 1990) and in male ddY mice (Matsumoto et al., 1997), compared to group-housed controls. However, a mild stressor (handling) has been shown to intensify the acute effects of ethanol on heart rate and body temperature in male rats (Peris & Cunningham, 1986); moreover, male rats exposed to the social stress of subordination display increased reactivity to the depressant effects of ethanol (Blakley & Pohorecky, 2006). Furthermore, others report that the sedative-hypnotic properties of ethanol are significantly enhanced in stressed male rats (Drugan et al., 1992) and C57BL/6 J male mice (Boyce-Rustay et al., 2007). Clearly, the actions of psychological stressors and glucocorticoids on sensitivity to the sedative effects of ethanol warrant further study and may be aided by a better understanding of the genetic factors that influence ethanol sensitivity.

The inbred long-sleep (ILS) and inbred short-sleep (ISS) mouse strains are an excellent resource for addressing the genetic bases of these behaviors, as they were derived from lines selected for differential central nervous system (CNS) sensitivity to a sedative dose of ethanol (McClearn & Kakihana, 1981). The noninbred progenitors of the ILS and ISS mice (the LS and SS mice, respectively) also differ in CORT release, with LS mice displaying higher plasma CORT levels following ethanol administration (Kakihana, 1976; Wand, 1990; Zgombick & Erwin, 1988), Corticotropin-releasing hormone (CRH) administration (Wand, 1990), and following exposure to psychological stress (Minnick et al., 1995; Wand, 1990) than their SS counterparts. Although the exact mechanism is not known (and in fact could be two independent events), these correlated responses to their original selection are consistent with the possibility that common genes may underlie the response to both stress and to ethanol.

The present study was designed to explore the relationship between stress, CORT levels, and ethanol sensitivity in ILS and ISS mice. Here, we tested the hypothesis that genetic differences in CORT release following psychological stress would alter subsequent sensitivity to the sedative-hypnotic effects of ethanol. We predicted that the ILS mice would display greater elevations in stress-induced CORT levels and that these elevations would subsequently cause a greater increase in sensitivity to ethanol than in the ISS mice.

Materials and methods

Subjects

Male and female ILS and ISS mice were bred and group housed in the specific pathogen-free facility at the Institute for Behavioral Genetics, Boulder, CO. The ILS and ISS show an eightfold difference in their ethanol sensitivity (as measured by loss of righting response after 4.1 g/kg ethanol), and have been widely used in alcohol research over the past 10 years. One to two weeks before testing, mice were moved to an adjacent building and allowed to acclimate. Animals were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.), and were given food and water ad libitum. Temperature was kept at a constant 22°C (±1). Animals of the same strain and sex were housed together at weaning, ~21–25 days of age; no singly housed mice were tested. Mice were between 60 and 100 days of age at the time of testing. All procedures were approved by the University of Colorado Institutional Animal Care and Use Committee, in accordance with National Institute of Health guidelines.

Psychological stress treatment

Testing took place between the hours of 0800 and 1200. Psychological stress consisted of taking mice from their home cage and placing them for 30 min in adjustable length, cylindrical Plexiglas tubes (1¹/₄ in diameter, 2–3¹/₄ in length) with air holes in the front, top, and back. Restraint took place in a separate room adjacent to the home-cage room. According to Herman and Cullinan (1997), this stressor is considered to be primarily psychological in nature because it does not produce pain or direct physical insult.

CORT pretreatment

The dose of CORT used for these experiments (1.25 mg/kg) was based on pilot studies and matched the elevation in plasma CORT levels in response to an acute-restraint stress session. CORT (Steraloids Inc, Catalog # Q1550) dissolved in vehicle (1% ethanol dissolved in 0.9% sterile saline in a 1:100 ratio) or vehicle alone was administered intraperitoneally (i.p.) 30 min prior to a 4.1 g/kg 20% wt:vol i.p. injection of ethanol.

LORE determination

Mice were tested for ethanol sensitivity by i.p. injection of ethanol (4.1 g/kg, 20% wt/vol solution in 0.9% sterile saline or 6.0 g/kg, 25% wt/vol solution in 0.9% sterile saline). Duration of LORE was determined using the method of McClearn and Kakihana (1981), modified as reported by Markel et al. (1997). Briefly, loss of the righting response occurred when the mouse could not right itself three times within 1 min. Upon LORE, mice were placed supine in a V-shaped sleep tray (~90° angle) and the time to regain the righting response was recorded. Any animal that failed to lose the righting reflex within 5 min was excluded from analysis. Mice with blood ethanol concentrations (BECs) two standard deviations from the mean were also excluded from the experiment. Regain of the righting reflex was defined as the ability of a mouse to right itself three times in 1 min (when a mouse righted itself, it was returned to a supine position in the trough). Upon regain of the righting response, 10 µl of blood were drawn from the retro-orbital sinus to determine BEC at regain of the righting response (BECRR).

Blood ethanol concentration

Ten microliters of retro-orbital blood were added to a 1.5 ml tube containing 200 μ l of perchloric acid on ice to precipitate blood solids. Following behavioral testing, tubes containing the blood samples were vortexed and centrifuged at 4,500 rpm for 10 min. The plasma or supernatant was then removed from the pellet and an equal volume of KOH was added to the supernatant to neutralize the perchloric acid. The sample was then vortexed and stored in the freezer until analysis (once per week). BEC was determined by spectrophotometric analysis using an ADH-linked enzyme assay as described by Smolen et al. (1986).

CORT enzyme immunoassay

Animals were sacrificed via rapid cervical dislocation and decapitation to collect trunk blood. Blood samples for the CORT assay were collected into 1.5 ml tubes containing 10 μ l of EDTA. Samples were spun at 10,000 rpm for 10 min and the supernatant collected. Measurement of plasma CORT was performed with the Assay Designs Correlate-EIA kit (catalog # 901-097) according to the manufacturer's protocol. Samples were analyzed simultaneously to avoid potential interassay variability. The intraassay coefficient of variation was 0.0635.

Experiment 1: Impact of acute- and repeated-restraint stress on duration of LORE (4.1 g/kg ethanol) and BECRR in ILS and ISS mice

Mice were equally divided into three treatment groups: restraint-naïve, acute-restraint, and repeated-restraint animals. Restraint-naïve animals (NO STRESS) were left alone in their home cages until the test day. Acute-restraint animals (ACUTE) received one 30-min session of restraint stress occurring immediately prior to testing; and repeated-restraint animals (REPEATED) received 5 consecutive days of 30-min restraint sessions, with the final (fifth) restraint session occurring immediately prior to testing. On test day, animals from each group were injected with 4.1 g/kg ethanol; LORE duration and BECRR were determined.

Experiment 2: Impact of acute-restraint stress on ethanol absorption and clearance in ILS and ISS mice

ILS and ISS mice were equally divided into NO STRESS and ACUTE treatment groups. ACUTE mice received a 4.1 g/kg i.p. injection of ethanol immediately following the restraint session, and their BEC was compared to unstressed ethanol-injected littermates. For the ethanol-absorption experiment, six 10- μ l blood samples were taken from the retro-orbital sinus at 1, 2, 3, 4, 5, and 15 min postethanol injection. It should be noted that it is possible there is a slight loss of accuracy taking retro-orbital blood samples at 1-min intervals. For the ethanol-clearance experiments, five 10- μ l blood samples were taken from the retro-orbital sinus of a separate cohort of mice at 30, 60, 120, 180, and 240 min postethanol injection.

Experiment 3: Impact of restraint stress on LORE (6.0 g/kg ethanol) and BECRR in ISS mice

ISS mice were injected with high-dose (6.0 g/kg, 25% wt/vol) ethanol and both the duration of LORE and BECRR were compared among NO STRESS, ACUTE, and REPEATED groups to control for any floor effects due to the decreased sensitivity to ethanol of ISS mice.

Experiment 4: Impact of acute- and repeated-restraint stress on CORT response in ILS and ISS mice

Mice were equally divided into three treatment groups; NO STRESS, ACUTE, and REPEATED, as described for experiment 1. Immediately after the final stress, animals from each group were sacrificed by cervical dislocation to collect trunk blood for CORT measurements.

Experiment 5: Impact of CORT preinjection on LORE (4.1 g/kg ethanol) and BECRR in ILS and ISS mice

The purpose of this experiment was to determine the impact of a CORT injection (which should produce CORT levels comparable to that seen following restraint stress) on ethanol sensitivity. Mice were injected (i.p.) with a 1.25 mg/kg injection of CORT or vehicle and mice were compared to noninjected littermates for LORE and BEC at awakening. This dose was based on pilot experiments (data not shown) to mimic the rise in CORT following 30 min of restraint stress. In the pilot experiment, CORT levels 30 min following CORT injection were not significantly different from CORT levels immediately following acute-restraint stress.

Data analysis

Initial analysis began with a factorial analysis of variance (ANOVA) to determine main effects, followed by Tukey HSD for post hoc analyses when appropriate. All analyses were conducted in SPSS v13.0 (SPSS Inc., Chicago, IL).

Results

Experiment 1: Impact of acute- and repeated-restraint stress on duration of LORE (4.1 g/kg ethanol) and BECRR in ILS and ISS mice

A three-way ANOVA showed main effects for strain ($F_{1, 173} = 1413.1$; $P < .0001$) and for treatment ($F_{2, 173} = 10.7$; $P < .0001$), but not sex, on LORE duration. Additionally, there was an interaction between strain and treatment on duration of LORE ($F_{2, 173} = 16.1$; $P < .0001$), thus the strains were analyzed separately. In the ILS mice, there was a significant effect of treatment on LORE duration ($F_{2, 85} = 14.7$; $P < .0001$). Post hoc analysis indicated that ILS mice displayed decreased LORE duration in the ACUTE ($P = .001$) and REPEATED ($P < .0001$) groups compared to their NO STRESS littermates (Fig. 1A). In the ISS mice, there was also a significant effect of treatment on LORE duration ($F_{2, 88} = 4.6$; $P = .012$). NO STRESS ISS mice had shorter durations of LORE compared to the REPEATED treatment ($P = .04$) and the REPEATED treatment group showed a significant increase in duration of LORE ($P = .01$) compared to the ACUTE treatment; however, no differences existed in LORE duration between NO STRESS and ACUTE treatment groups in ISS mice (Fig. 1A).

Additionally, the effects of strain, sex, and treatment on BECRR were examined. A three-way ANOVA found main effects for strain ($F_{1, 173} = 55.9$; $P < .0001$), and treatment ($F_{2, 173} = 6.2$; $P = .002$) but not sex on BECRR, as well as a strain by treatment interaction ($F_{2, 173} = 4.7$; $P = .010$). Thus, the strains were analyzed separately. In the ILS mice, a one-way ANOVA showed a main effect of treatment ($F_{2, 85} = 12.1$; $P < .0001$), on BECRR (Fig. 1B). Post hoc analysis revealed that BECRR was significantly elevated in ACUTE ILS mice compared to the NO STRESS ($P < .0001$) and REPEATED ($P = .02$) treatment groups. In the ISS mice, a one-way ANOVA showed a main effect of treatment ($F_{2, 88} = 3.2$; $P = .044$). ISS mice in the NO STRESS treatment had significantly increased ($P = .043$) BECRR compared to the REPEATED treatment groups.

Experiment 2: Impact of acute-restraint stress on ethanol absorption and clearance in ILS and ISS mice

A three-way ANOVA found main effects of strain ($F_{1, 510} = 5.45$; $P = .020$) and of treatment ($F_{1, 510} = 13.4$; $P < .0001$), but not sex on the rate of ethanol absorption in ILS and ISS mice. ISS mice had lower absorption rates than ILS animals, and in both strains, stressed mice had decreased rates of ethanol absorption compared to unstressed littermates (Fig. 2A, B). However, these differences dissipated over time, as an ANOVA found no significant effects of strain or treatment on ethanol elimination in ILS or ISS mice (Fig. 2C, D). A linear regression

shows that stressed ILS mice cleared ethanol at a rate of 89.4 mg% per hour compared to unstressed ILS mice, which had a clearance rate of 84.6 mg% per hour; yet a *t*-test on clearance rates showed these differences to be nonsignificant ($P = .43$). Stressed ISS mice cleared ethanol at a rate of 111.6 mg% per hour compared to unstressed ISS mice, which had a clearance rate of 114 mg% per hour; a *t*-test found no significant differences between these clearance rates ($P = .71$). The differences observed in clearance rates between strains failed to reach significance across either treatment.

Experiment 3: Impact of acute- and repeated-restraint stress on duration of LORE (6.0 g/kg ethanol) and BECRR in ISS mice

To control for floor effects (due to decreased sensitivity to ethanol), and to produce an equivalent duration of LORE to their ILS counterparts, a separate cohort of ISS mice were injected with 6.0 g/kg, 25% wt/vol ethanol and the duration of LORE was compared between NO STRESS, ACUTE, and REPEATED treatment groups. A two-way ANOVA showed significant main effects for both sex ($F_{1, 70} = 6.4$; $P = .01$) and treatment ($F_{2, 70} = 9.3$; $P < .0001$) on LORE duration (Fig. 3A). Additionally, the effects of sex and treatment on BECRR were examined. A two-way ANOVA found main effects for sex ($F_{1, 68} = 5.8$; $P = .019$), and treatment ($F_{2, 68} = 4.4$; $P = .017$) on BECRR, as well as a sex by treatment interaction ($F_{2, 68} = 4.8$; $P = .011$). ISS mice in the REPEATED group showed a significant increase in BECRR compared to both ACUTE ($P = .016$) and NO STRESS littermates ($P = .045$, Fig. 3B).

Experiment 4: Impact of acute- and repeated-restraint stress on CORT response in ILS and ISS mice

ILS and ISS mice were evaluated for their CORT response to acute and repeated restraint to determine if they displayed similar patterns of HPA axis activation following stress. A three-way ANOVA revealed a main effect for strain ($F_{1, 121} = 5.8$; $P = .02$) and for treatment ($F_{2, 121} = 153.0$; $P < .0001$), but not sex on CORT levels in the mice, as well as a strain by treatment interaction ($F_{2, 121} = 6.7$; $P = .02$). As a result, the strains were analyzed separately. In the ILS, a one-way ANOVA found a main effect of treatment on CORT levels ($F_{2, 60} = 79.9$; $P < .0001$). Post hoc analysis indicated that the NO STRESS animals had five-fold to tenfold lower CORT than both the ACUTE ($P < .0001$) and the REPEATED ($P < .0001$) treatment groups (Fig. 4). In addition, the ACUTE treatment group showed significantly higher CORT levels than the REPEATED treatment group ($P < .0001$). In the ISS mice, a one-way ANOVA also found a main effect of treatment on CORT levels ($F_{2, 61} = 69.8$; $P < .0001$), yet here, the NO STRESS animals had significantly lower CORT compared to both the ACUTE ($P < .0001$) and REPEATED ($P < .0001$) animals; yet, mice exposed to ACUTE and REPEATED treatments did not differ from each other (Fig. 4). There were no differences between the ILS and ISS in the NO STRESS or ACUTE treatment groups. However, there was a difference between strains in the REPEATED treatment group ($F_{1, 37} = 8.8$; $P = .005$), with ISS mice displaying higher CORT levels than ILS mice (Fig. 4).

Experiment 5: Impact of CORT pretreatment on duration of LORE (4.1 g/kg ethanol) and BECRR in ILS and ISS mice

To determine if the effects of restraint on LORE duration and BECRR could be mediated by CORT alone, ILS and ISS mice were preinjected with either a saline vehicle or CORT 30 min prior to testing and compared to noninjected littermates. There were significant effects for strain ($F_{1, 103} = 516.54$; $P < .0001$), sex ($F_{1, 103} = 5.86$; $P = .017$), and treatment ($F_{2, 103} = 3.2$; $P = .045$) on duration of LORE in ILS and ISS mice (Fig. 5A). Upon visual inspection of the data, it appeared that the ISS mice receiving a CORT injection displayed a decreased duration of LORE. Subsequently, a one-way ANOVA was performed to determine if CORT treatment

had an effect in the ISS. This found an effect for treatment on LORE duration ($F_{2,45} = 10.82$; $P < .0001$). Post hoc analysis revealed that ISS mice receiving a pretreatment of CORT had a decrease in LORE duration compared to their noninjected ($P < .0001$) and saline-injected controls ($P = .001$). A one-way ANOVA found no significant effects of treatment on LORE duration in ILS mice.

Additionally, a three-way ANOVA found main effects for strain ($F_{1,100} = 105.22$; $P < .0001$) and sex ($F_{1,100} = 26.31$; $P < .0001$), but not treatment on BECRR (Fig. 5B).

Discussion

Initial sensitivity to the intoxicating effects of ethanol is a genetically mediated trait. Using an animal model, these results support this finding and suggest that in addition to genetic susceptibility, environmental factors (e.g., restraint stress, CORT administration) also influence sensitivity to the sedative-hypnotic effects of ethanol, albeit in divergent directions and in a genotype-dependent manner. In ILS mice, the more ethanol sensitive of the genotypes, acute and repeated restraint stress decreased sensitivity to the sedative-hypnotic effects of ethanol as measured by LORE duration. The increase in BECRR in the ILS ACUTE group suggests that a decrease in CNS sensitivity underlies the shortened LORE duration in the ACUTE treatment; however, CNS sensitivity does not fully account for the shortened LORE duration in the REPEATED group because BECRR did not change. In the ISS mice (the ethanol-insensitive genotype), repeated restraint stress caused an increase in sensitivity to ethanol at the 4.1 g/kg dose as indicated by increased LORE duration and decreased BECRR. This effect was contrary to that of the ILS mice. When ISS mice were given a higher dose of ethanol, the results were less clear. At the 6.0 g/kg dose, ISS mice showed a similar increase in duration of LORE after repeated stress as they did at the 4.1 g/kg dose. However, their BECRR was also higher than the NO STRESS and ACUTE STRESS groups, thus suggesting a decrease in sensitivity. As Radcliffe et al. (2005) have demonstrated, ISS mice display a large drop in body temperature in response to a 6 g/kg dose of ethanol, and it is possible that the high dose of ethanol induced severe hypothermia in the ISS, thus decreasing their rate of metabolism; that is, the ISS became less sensitive, but the metabolic effect caused a longer LORE duration. It is also feasible that at such a high dose, the increased BECRR in the ISS may have been caused by the development of acute functional tolerance. However, little is known of the effect of acute or repeated stress on acute tolerance.

Duration of LORE is due to the effects of both CNS ethanol sensitivity and pharmacokinetic parameters such as ethanol absorption and clearance. A change in either will alter the duration of LORE, although the latter would probably have a much greater effect than the former after i.p. administration. Although LORE and BECRR are generally expected to be correlated, BEC is a more sensitive indicator of the effects of ethanol in the CNS. To determine if changes in metabolism were causing the differences in LORE duration, ethanol absorption and clearance experiments were performed on acutely stressed and unstressed ILS and ISS mice. A significant decrease in ethanol absorption was observed in acutely stressed mice, amounting to about a 10% difference in BEC values. However, a three-way ANOVA found no significant differences in ethanol clearance across treatments, thus suggesting that pharmacokinetic parameters were not responsible for the effects of acute stress on waking blood ethanol values. Although the linear regression showed modest strain differences in slope and BEC at time = 0, there were no differences in ethanol elimination between stress treatments. The slight differences in ethanol metabolism between strains have been reported before (Smolen et al., 1986) and might marginally contribute to the overall differences in sensitivity; however, acute stress had no significant effect on clearance and is not sufficient to explain the behavioral differences that were observed. In addition, it is possible that repeated stress could alter ethanol absorption or clearance in ILS and ISS mice. Unfortunately, the emergence of breeding problems in the

colony at the Institute for Behavioral Genetics has severely limited the availability of these strains of mice, making them unavailable for additional testing. Further study on the effects of repeated stress on ethanol pharmacokinetics would be informative.

Because of the observed differences between ILS and ISS in their LORE and BECRR following restraint stress, the mice were next examined to determine if they differed in their CORT responsiveness to restraint stress. This is the first time the ISS and ILS mice have been examined for differences in CORT responses following psychological stress (previous work has used the outbred SS and LS only). Minnick et al. (1995) reported that stressed LS mice released significantly more CORT than stressed SS mice; however, our findings do not concur with their observation. Here, ILS and ISS mice did not differ in plasma CORT levels immediately following acute-restraint stress. However, measurement of CORT levels at various time points following restraint would provide important information on how the strains adapt to repeated-restraint stress. The fact that our CORT results in ILS and ISS mice do not follow the same relative patterns as those seen in the progenitor strains does not necessarily imply that these differences in CORT responsiveness are a result of inbreeding; rather, they may be a function of the different stressors used by Minnick et al. (45 min of exposure to the open arm of an Elevated Plus Maze). Additionally, results indicated that the CORT response of the ILS mice began to habituate to repeated-restraint stress, whereas the CORT response of the ISS mice did not (Fig. 4). Further research on the effects of repeated stress and CORT habituation in ISS mice would be informative.

Previous studies in rodents suggest that exposure to stress may modulate sensitivity to ethanol, yet the ways in which this occurs remain unclear. In experiment 5, we sought to determine if enhanced CORT levels alone (in the absence of the psychological stress component) were sufficient to alter sensitivity to the sedative-hypnotic effects of ethanol.

Interestingly, there were no effects of saline or CORT injections on LORE duration in ILS mice compared to their noninjected littermates (Fig. 5A). However, ISS mice injected with CORT showed a decrease in duration of LORE compared to both saline and non-injected littermates (Fig. 5A). There were no effects of treatment on BECRR in either strain, thus suggesting that some property of ethanol pharmacokinetics was altered in the ISS, rather than a change in CNS sensitivity. Interestingly, the effect of CORT injection on LORE duration is in the opposite direction to the effect of restraint stress in ISS mice, and may be due to a variety of reasons. One explanation for these strain differences may be that the elevated CORT levels caused by the CORT injections dissipated in the ILS mice before having the opportunity to alter LORE duration due to their inherently longer sleep times. However, this possibility is unlikely, because the exogenous CORT injections were matched to mimic the rise in CORT following 30 min of restraint stress (which did have a significant effect on the LORE duration of ILS mice).

Alternatively, it is feasible that other aspects of activated neurocircuitry following psychological stress are primarily responsible for the influence of stress on LORE independent of elevated CORT concentrations. In fact, numerous labs have shown that genetic differences between ILS and ISS in 5HT, DA, norepinephrine, GABA, and NMDA (Hanania et al., 2000, 2004; Hanania & Zahniser, 2002; Haughey et al., 2005; Proctor et al., 2004; Zahniser et al., 1992) receptors may be responsible for habituation (or lack thereof) of the HPA-axis response to stress and underlie their differing sensitivity to ethanol following stress or glucocorticoid injection. Further research using the ILS and ISS strains to determine how these neural systems are differentially regulated by stress exposure might prove fruitful in further elucidating genes and gene networks involved in stress and ethanol sensitivity.

In conclusion, the present study demonstrated that exposure to restraint stress and exogenous CORT injection modified the sedative-hypnotic effects of ethanol on ILS and ISS mice in different ways and in a genotype-dependent fashion. This suggests that differences in CORT levels alone are not sufficient to explain the influence of stress on sensitivity to the sedative-hypnotic effects of ethanol; and further research on the neuroanatomical, biochemical, and molecular mechanisms underlying this phenomenon are needed. Ongoing studies are being conducted to continue examining the effects of restraint stress on other ethanol sensitivity-related phenotypes (such as the hypothermic, locomotor activating, or anxiolytic effects of ethanol) across inbred, congenic, and mutant strains of mice. These will be useful in further exploring the impact of stress-induced alterations of critical responses to ethanol using a mouse model.

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Fig. 1.

(A, B) Impact of acute- and repeated-restraint stress on duration of loss of the righting reflex (LORE) (4.1 g/kg ethanol) and blood ethanol concentration at regain of the righting response in inbred long-sleep (ILS) and inbred short-sleep (ISS) mice. Male and female mice were divided into three treatment conditions: restraint-naïve animals (NO STRESS) (ISS $n = 30$, ILS $n = 32$), acute-restraint animals (ACUTE) (ISS $n = 34$, ILS $n = 29$), and repeated-restraint animals (REPEATED) (ISS $n = 24$, ILS $n = 24$). On the test day, animals from each group were injected with 4.1 g/kg ethanol; duration of LORE was recorded and blood ethanol concentration at regain of the righting response was measured. Symbols indicate significant within-strain effect of treatment (* $P < .05$ as compared to NO STRESS, ** $P < .001$ as compared to NO STRESS, *** $P < .0001$ as compared to NO STRESS, # $P < .05$ as compared to ACUTE, ^ $P < .05$ as compared to REPEATED). Error bars equal to ± 1 standard error of the mean.

Fig. 2.

Stress effects on ethanol absorption and clearance in inbred short-sleep (ISS) and inbred long-sleep (ILS) mice. (A, B) Stress effects on ethanol absorption. Male and female ISS (A) and ILS (B) mice were equally divided into restraint-naïve animals (NO STRESS) (ISS $n = 20$, ILS $n = 21$) and acute-restraint animals (ACUTE) (ISS $n = 19$, ILS $n = 25$) treatments. Mice received a 4.1 g/kg i.p. injection of ethanol and blood ethanol concentration was compared between stressed and unstressed littermates at 1, 2, 3, 4, 5, and 15 min postethanol injection. Error bars equal to ± 1 standard error of the mean. (C, D) Stress effects on ethanol clearance. Male and female ISS (C) and ILS (D) mice were equally divided into NO STRESS (ISS $n = 14$, ILS $n = 19$) and ACUTE treatments (ISS $n = 16$, ILS $n = 19$). Mice received a 4.1 g/kg i.p. injection of ethanol and blood ethanol concentration was compared between stressed and unstressed littermates at 30, 60, 120, 180, and 240 min postethanol injection. Error bars equal to ± 1 standard error of the mean.

Fig. 3. (A, B) Impact of acute- and repeated-restraint stress on duration of loss of the righting reflex (LORE) (6.0 g/kg ethanol) and blood ethanol concentration at regain of the righting response in inbred short-sleep (ISS) mice. Male and female ISS mice were divided into three treatments: restraint-naïve animals (NO STRESS) ($n = 27$), acute-restraint animals (ACUTE) ($n = 26$), and repeated-restraint animals ($n = 16$). On test day, animals from each group were injected with 6.0 g/kg ethanol; duration of LORE and BECRR was measured. Symbols indicate significant within-strain effect of treatment (* $P < .05$ as compared to NO STRESS, ### $P < .0001$ as compared to ACUTE, # $P < .05$ as compared to ACUTE). Error bars equal to ± 1 standard error of the mean.

Fig. 4.

The effects of acute- and repeated-restraint stress on corticosterone (CORT) levels in inbred long-sleep (ILS) and inbred short-sleep (ISS) mice. Male and female mice were divided into three treatments: restraint-naïve animals (NO STRESS) (ILS $n = 24$, ISS $n = 26$), acute-restraint animals (ACUTE) (ILS $n = 17$, ISS $n = 17$), and REPEATED (ILS $n = 19$, ISS $n = 18$). On test day, animals from each treatment were sacrificed by cervical dislocation to collect trunk blood for CORT measurements. Symbols indicate significant within-strain effect of treatment (*** $P < .0001$ as compared to NO STRESS, ### $P < .0001$ as compared to ACUTE). Error bars equal to ± 1 standard error of the mean.

Fig. 5.

(A, B) Impact of corticosterone (CORT) pretreatment, instead of restraint stress, on duration of loss of the righting reflex (LORE) (4.1 g/kg ethanol) and blood ethanol concentration at regain of the righting response (BECRR) in inbred short-sleep (ISS) and inbred long-sleep (ILS) mice. Mice were injected with a 1.25 mg/kg dose of CORT (ISS $n = 15$, ILS $n = 19$) or saline (ISS $n = 15$, ILS $n = 19$) and compared to noninjected littermates (ISS $n = 18$, ILS $n = 17$) for duration of LORE and BECRR. Symbols indicate significant within-strain effect of treatment (*** $P < .0001$ as compared to restraint-naïve animals, ### $P = .0001$ as compared to acute-restraint animals).