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Escalation of Intake Under Intermittent Ethanol Access in Diverse Mouse Genotypes

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Abstract

Experimental animals offered continuous 24-hour free choice access to ethanol rarely display voluntary ethanol consumption at levels sufficient to induce intoxication or to engender dependence. One of the simplest ways to increase voluntary ethanol intake is to impose temporal limitations on ethanol availability. Escalation of ethanol intake has been observed in both rats and mice under a variety of different schedules of alternating ethanol access and deprivation. While such effects have been observed in a variety of rat and mouse genotypes, little is known concerning possible genetic correlations between responses to intermittent ethanol access and other ethanol-related phenotypes. In the present study, we examined the effects of intermittent ethanol access in mouse genotypes characterized by divergent responses to ethanol in other domains, including ethanol preference (C57BL/6J and C3H/HeJ mice), binge-like ethanol drinking (HDID-1 and HS/Npt mice), and ethanol withdrawal severity (WSP-2 and WSR-2 mice). While intermittent ethanol access resulted in escalated ethanol intake in all tested genotypes, the robustness of the effect varied across genotypes. On the other hand, we saw no evidence that the effects of intermittent access are correlated with either binge-like drinking or withdrawal severity, and only weak evidence for a genetic correlation with baseline ethanol preference. Thus, these different ethanol-related traits appear to depend on largely unique sets of genetic mediators.

Keywords

binge drinking; ethanol; intermittent ethanol access; mouse; selective breeding; withdrawal

Introduction

Considerable research has been aimed at identification of the behavioral, physiological and genetic factors regulating free-choice (i.e., 2-bottle) ethanol intake under conditions of

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continuous 24-hour access. Nevertheless, the clinical relevance of such studies is limited by the fact that rats and mice rarely achieve intoxicating blood ethanol levels during free-choice drinking, nor do they typically show signs of dependence when ethanol access is terminated (Crabbe et al., 2010). Thus, 24-hour free-choice ethanol intake does not provide an adequate animal model for either binge-like or dependence-driven ethanol intake, as expressed in human alcohol abuse disorders (Crabbe, 2008; Crabbe et al., 2011).

In recognition of this limitation, researchers have developed a number of procedures designed to engender “excessive” levels of voluntary ethanol intake that might more closely resemble problem drinking in humans. Such procedures include the use of sweetened ethanol solutions, as well as “fading” procedures in which the concentrations of ethanol and/or sweetener are gradually adjusted over time, while other procedures make use of food and/or water deprivation to increase ingestive motivation. One of the simplest ways to increase voluntary ethanol intake, however, is to impose temporal limitations on ethanol availability (Breese et al., 2005; Sanchis-Segura and Spanagel, 2006; Crabbe et al., 2011). For example, Sinclair and Senter (1967, 1968) showed that rats with a prior history of free-choice ethanol drinking displayed a dramatic but temporary increase in ethanol intake following a 1-week period of ethanol deprivation, a phenomenon they termed the “alcohol deprivation effect” (ADE). While initial attempts to demonstrate the ADE in mice were not successful (Geiger and Barker, 1976), subsequent research has shown that the ADE may be expressed in diverse rat (Heyser et al., 1997; Spanagel and Holter, 1999; Rodd-Henricks et al., 2000a, b; Serra et al., 2003) and mouse (Salimov et al., 2000; Khisti et al., 2006; Zghoul et al., 2007; Tambour et al., 2008; Sparta et al., 2009) genotypes. Thus, the ADE is considered to provide a plausible animal model of excessive, relapse-like drinking (Sanchis-Segura and Spanagel, 2006), as is commonly observed in human alcohol abuse. The earliest ADE studies showed that the magnitude and duration of the ADE is sensitive to both the duration of ethanol adaptation prior to deprivation (Sinclair and Senter, 1968) and to the duration of deprivation (Sinclair, 1972), while more recent studies demonstrate that the ADE may also be potentiated by repeated cycles of ethanol access and deprivation (i.e., the “repeated alcohol deprivation effect” or RADE) (Spanagel and Holter, 1999; Rodd-Henricks et al., 2000 a, b). The RADE may reflect neuroadaptive changes associated with the transition from alcohol abuse to alcohol addiction (Breese et al., 2005; Sanchis-Segura and Spanagel, 2006; Crabbe et al., 2011).

Wise (1973) developed a procedure that appears conceptually similar to the RADE in which rats are maintained under intermittent (every-other-day; EOD) 24-hour ethanol access. Indeed, the main difference between the EOD and RADE procedures is the more rapid alternation between drinking and non-drinking days in the EOD procedure. The EOD procedure (or slight variants thereof) has been shown to reliably increase daily ethanol intake in both rats (Wise, 1973; Simms et al., 2008; Hargreaves et al., 2009; Loi et al., 2010) and mice (Melendez, 2011; Hwa et al., 2011). Similarly, Melendez et al. (2006) demonstrated escalation of ethanol intake in mice under a one-day-per-week intermittent access schedule. Although studies using intermittent 24-hour ethanol access procedures have generally avoided the “ADE” terminology (but see Melendez et al., 2006), it seems likely that exposure to repeated periods of ethanol deprivation is critical for the elevated ethanol intake seen in both RADE and intermittent-access procedures, as suggested previously (Simms et al., 2008; Hwa et al., 2011). Indeed, both RADE and EOD procedures induce at least some animals to voluntarily consume sufficient ethanol to achieve mildly intoxicating blood ethanol levels and to display behavioral signs of ethanol dependence (Bell et al., 2008; Holter et al., 2000; Simms et al., 2008; Hwa et al., 2011).

Several rat genotypes have been tested under ADE and/or EOD procedures, and preliminary indications are that sensitivity to these procedures varies with genetic background. For

example, while P rats showed an ADE following a single deprivation, HAD1 and HAD2 rats expressed an ADE only after multiple deprivations (Rodd-Henricks et al., 2000a, b). Further, repeated deprivations potentiated the expression of the ADE in both P and HAD-2 rats but not in HAD-1 or Sardinian alcohol-preferring (sP) rats, despite the fact that all these lines were selectively bred for the same high ethanol-preference phenotype (Rodd-Henricks et al., 2000a, b; Serra et al., 2003). In mice, animals of the relatively lower-drinking C57BL/6NCrl substrain showed more robust ADE and RADE effects than mice of the higher-drinking C57BL/6J substrain, suggesting that the magnitude of the ADE may be negatively correlated with initial ethanol preference (Khisti et al., 2006). Strain differences have also been observed under the EOD access procedure, which resulted in much more robust acceleration of ethanol intake in low-to-moderate drinking Wistar and Long-Evans outbred rats relative to high-drinking P rats (Simms et al., 2008). While the relative insensitivity of the P rat to EOD access could reflect in part a ceiling effect in this very high-drinking line, the P rat is capable of displaying a robust RADE (Rodd-Henricks et al., 2000a), suggesting that baseline ethanol preference may not be an absolute limiting factor in these procedures.

While a diversity of rat genotypes have been tested under EOD access, to date intermittent-access procedures have been employed only in C57BL/6 mice. The present study was therefore designed to explore the effects of intermittent access on voluntary ethanol intake in a range of mouse genotypes characterized by diverse ethanol-related phenotypes. In Experiment 1, we assessed free-choice ethanol intake in high-drinking C57BL/6J and low to moderate-drinking C3H/HeJ inbred mice (Rodgers and McClearn, 1962; Belknap et al., 1993) under continuous and intermittent, 1 day per week (IA1) conditions. This experiment further examined the effects of baseline ethanol preference on responsiveness to intermittent ethanol access. In Experiment 2, we assessed High Drinking-in-the-Dark (HDID-1) and genetically heterogeneous HS/Npt control mice under continuous, IA1 and 3 day per week (IA3) access conditions. HDID-1 mice were selectively bred from HS/Npt stock for achieving high blood ethanol concentrations (BECs) in the Drinking-in-the-Dark (DID) protocol, a procedure that engenders binge-like drinking to intoxication (Crabbe et al., 2009). Despite the dramatic difference between HDID-1 and HS/Npt mice in the DID protocol, these two lines show similar levels of 24-hour free-choice ethanol intake (Crabbe et al., 2011). Thus, Experiment 2 tested the hypothesis that alleles contributing to DID drinking also influence the response to intermittent ethanol access. Finally, Experiment 3 compared the responses of Withdrawal-Seizure-Prone (WSP-2) and Withdrawal-Seizure-Resistant (WSR-2) mice to both IA1 and IA3 procedures. WSP and WSR mice were selectively bred from genetically heterogeneous HS/Ibg stock using bidirectional selection for high and low sensitivity to ethanol withdrawal, as indexed by the handling-induced convulsions (HIC) test (Kosobud and Crabbe, 1986; Crabbe and Phillips, 1993). Despite their dramatically different withdrawal sensitivity, little is known of regarding ethanol preference in WSP and WSR mice. While Kosobud et al. (1988) reported that WSR-2 mice (i.e., the lineage employed in the present experiment) consumed more ethanol than WSP-2 (or WSR-1) mice, more recent observations (Crabbe et al., unpublished) have failed to detect significant differences in preference drinking among WSP and WSR mice, suggesting that genetic drift may have occurred over the long history of these lines. Thus, Experiment 3 tested the hypothesis that alleles related to ethanol withdrawal sensitivity also contribute to responsiveness to intermittent ethanol access.

Beyond the exploration of multiple genotypes, a second objective of this study was to examine the possible modulation of intermittent access effects by two different environmental conditions – photoperiod duration and running-wheel access – previously shown to affect ethanol intake under continuous-access conditions (photoperiod: Geller, 1971; Geller and Hartmann, 1977; Millard and Dole, 1983; Goodwin et al., 1999; Rosenwasser et al., 2010; running-wheel access: McMillan et al., 1995; Ozburn et al., 2008;

Ehringer et al., 2009; Hammer et al., 2010). In Experiment 1, half the animals in each genotype were maintained under a short (light-dark 6:18) and half under a long (light-dark 18:6) photoperiod. In Experiments 2 and 3, half the animals of each genotype were allowed continuous access to running wheels and half were housed in identical cages with locked running wheels.

Experiment 1

Materials and Methods

Animals and apparatus—Male C57BL/6J (B6) and C3H/HeJ (C3H) mice (N = 15 per strain) were obtained from The Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age. Upon arrival in the laboratory, mice were placed individually in standard mouse cages equipped with running wheels (wheel diameter: 11.5 cm; Coulbourn Instr., Whitehall, PA). Running-wheel cages were housed three per shelf in light- and sound-attenuating cabinets, and illumination was provided by standard fluorescent tubes. Wheel-turns were monitored via microswitch and stored in 1-minute bins using the ClockLab computer interface system (Coulbourn Instr., Whitehall, PA), which also controlled light-dark cycles within the cabinets. Food (Prolab RMH 3000) was continuously available throughout the experiment, while fluids were provided as described below.

Procedures—Half of the animals of each strain were maintained throughout the experiment under a long-photoperiod light-dark cycle comprising 18 hours of light and 6 hours of darkness (LD 18:6) and the other half were maintained under a short photoperiod (LD 6:18). Following an initial 2-week acclimation period, mice were provided continuous access to both plain tap water and 10% v/v ethanol solution via separate bottles equipped with standard ball-point sipper tubes. During this phase of the experiment, water and ethanol intakes were determined at weekly intervals, and bottle positions were switched following each measurement. After 6 weeks of free-choice preference drinking, all mice were exposed to 6 weeks of intermittent ethanol access, during which ethanol was removed and mice were offered two bottles of tap water for 6 days, followed by a single 24-hour period of free-choice preference drinking. Water and ethanol intakes were determined for each of the 6 free-choice drinking days. Finally, the mice were returned to continuous free-choice ethanol access for an additional 6 weeks, and measurements were made as described above. Body weights were recorded following each weekly intake determination throughout the 18 weeks of the experiment.

Data analysis—Ethanol intakes were expressed as grams of ethanol per kg of body weight per day, while water intakes were expressed as ml per mouse per day. In addition, ethanol preference ratios were computed as the volume of 10% ethanol divided by the total consumed fluid volume (ethanol plus water). Data are presented graphically on a week-by-week basis and were collapsed into three 6-week blocks (first continuous access, intermittent access, second continuous access) for statistical analysis using a 3-way factorial ANOVA, with strain and photoperiod as between-groups factors and experimental phase as a repeated-measures factor. Follow-up pairwise comparisons between experimental phases within strains were performed using Bonferroni-protected dependent-samples t-tests.

Results

Ethanol intake data are presented on a week-by-week basis in Fig. 1, and grouped into the three experimental phases for statistical analysis in Fig. 2. As expected, B6 mice consumed more ethanol than C3H mice throughout the entire experiment (main effect of strain: $F(1,26) = 109.230$, $p < 0.001$). While both strains displayed escalated ethanol intake during intermittent access (main effect of experimental phase: $F(2,52) = 503.741$, $p < 0.001$), this

effect was more robust in C3H mice, which showed an approximate 4-fold increase, relative to B6 mice, which showed an approximate doubling of ethanol intake per drinking day (strain by experimental phase interaction: $F(2,52) = 18.125$, $p < 0.001$). Increased ethanol intake appeared to reach asymptotic levels by the second week of intermittent access in both strains (Fig. 1). When animals were returned to continuous access, ethanol intake in C3H mice returned to initial levels by the second week, but was persistently lower during the second continuous access phase than during the first in B6 mice, resulting in a relative attenuation of the normal strain difference during the second continuous-access phase (Figs. 1, 2). Thus, pairwise comparisons indicated that ethanol intake was significantly lower in B6 mice and slightly but significantly higher in C3H mice during the second continuous-access phase relative to the first. Finally, both strains showed an unexpected pattern comprising alternating weeks of relatively high and relatively low intake during intermittent ethanol access (Fig. 1). We believe that this alternating pattern may have been due to the relative proximity of the two drinking tubes to the cage-top food hopper, which alternated weekly. Indeed, this pattern was not seen in Experiments 2 and 3, when we ensured that food was available in equal proximity to both drinking tubes.

Data for ethanol preference ratios were very similar to those described above, both on a week-by-week basis (data not shown) and across experimental phases (Fig. 2). ANOVA again revealed significant main effects of strain ($F(1,26) = 115.496$, $p < 0.001$) and experimental phase ($F(2,52) = 111.086$, $p < 0.001$), as well as a significant strain by phase interaction ($F(2,52) = 10.106$, $p < 0.001$). Pairwise comparisons indicated that, as for ethanol intake, ethanol preference in C3H mice was significantly higher during the second continuous-access phase relative to the first, but in contrast to ethanol intake, ethanol preference in B6 mice was not significantly lower in the second continuous-access phase relative to the first.

Analysis of water intake showed that C3H mice consumed more water than B6 mice ($F(1,26) = 27.050$, $p < 0.001$) (Fig. 2). In addition, water intake also showed a significant main effect of experimental phase ($F(2,52) = 6.022$, $p = 0.004$) and a strain by phase interaction ($F(2,52) = 8.237$, $p = 0.001$). Nevertheless, pairwise comparisons indicated that this strain-by-phase interaction was due entirely to a significant reduction in water intake in the second continuous-access phase relative to both the first continuous-access phase and the intermittent-access phase, seen only in C3H mice. Thus, the escalation of ethanol intake and ethanol preference seen under intermittent ethanol access was specific to ethanol, and was not seen for water intake.

Despite the robust effects of strain and ethanol access conditions, there were no significant main effects or interactions involving photoperiod on any of the intake measures. Nevertheless, given prior positive findings of photoperiodic modulation of ethanol intake, we performed an exploratory analysis examining possible photoperiod effects on overall ethanol and water intake within strains (Fig. 3). While this analysis failed to detect any significant effects of photoperiod in B6 mice, C3H mice maintained under long-photoperiod conditions (LD 18:6) showed significantly elevated ethanol intake ($t(13) = 2.357$, $p = 0.035$) and ethanol preference ($t(13) = 2.293$, $p = 0.039$) relative to those maintained in LD 6:18.

Experiment 2

Materials and Methods

Animals and apparatus—Male HDID-1 and HS/Npt mice ($N = 15$ HDID-1, 14 HS/Npt) were obtained from the breeding facilities at the Oregon Health and Science University at about 8 weeks of age and upon arrival were placed individually into commercial running-wheel cages. All details concerning animal housing were identical to those described for

Experiment 1, except that the running wheels were mechanically locked for half of the animals in each breeding line.

Procedures—The first three phases of this experiment were identical to those described above for Experiment 1, comprising 6 weeks of continuous free-choice access to 10% ethanol, followed by 6 weeks of intermittent, IA1 conditions, and then by an additional 6 weeks of continuous ethanol access. These conditions were then followed by 6 weeks under a schedule of intermittent, 3 days per week (IA3) ethanol access, during which 24-hour access periods began each Monday, Wednesday and Friday, after which animals were returned to continuous ethanol access for the final 6 weeks of the experiment. As in Experiment 1, ethanol and water intakes and body weights were recorded on a weekly basis during continuous ethanol access, and following each 24-hour access period under both IA1 and IA3 intermittent ethanol access. Under IA3 conditions, intake data were collapsed into weekly time bins and then into 6-week experimental phases for statistical analysis

Data analysis—Analytic and statistical procedures were identical to those described for Experiment 1 except that the between-groups ANOVA factors were breeding line and running-wheel access, and the repeated-measures factor comprised five rather than three consecutive experimental phases.

Results

Fig. 4 shows weekly ethanol intake data for the entire experiment, while Fig. 5 shows mean ethanol intake, ethanol preference, and water intake for each of the five 6-week phases of the experiment. Ethanol intake showed an approximate 2.5-fold increase under both intermittent-access conditions relative to continuous ethanol access in both HDID-1 and HS/Npt lines. ANOVA revealed a significant main effect of experimental phase ($F(4,100) = 198.992$, $p < 0.001$) on ethanol intake, but no significant main effects of either breeding line or wheel access, nor any significant interactions. Similarly, ANOVA detected only a significant main effect of experimental phase on ethanol preference ($F(4,100) = 156.208$, $p < 0.001$), but no other significant effects. While both ethanol intake and ethanol preference were slightly higher in HDID-1 mice relative to HS/Npt mice throughout the experiment (Figs. 4, 5), this difference did not reach significance for either variable. Bonferroni-protected pairwise comparisons between experimental phases showed that ethanol intake differed between every pair of conditions other than between the second and third continuous-access phase, which were both significantly lower than the first continuous-access phase. Similarly, ethanol preference differed between all pairs of conditions other than the second and third continuous-access phase and the two intermittent-access phases. Thus, while ethanol intake was slightly but significantly lower during IA3 relative to IA1 conditions, this difference was not seen in ethanol preference. Both ethanol intake and ethanol preference however were significantly lower during the second and third continuous-access phase relative to the first.

Analysis of water intake revealed significant main effects of experimental phase ($F(4,100) = 67.228$, $p < 0.001$) and running-wheel access ($F(1,25) = 32.247$, $p < 0.001$), as well as a phase by breeding line ($F(4,100) = 4.486$, $p = 0.002$) interaction (Fig. 5). Despite this interaction, pairwise tests showed no significant differences in water intake between the two breeding lines for any of the 5 experimental phases. Comparison of water intake between experimental phases showed that water intake was significantly different between all pairs of phases other than the fact that water intake in the second continuous-access phase did not differ from either the IA3 phase or the third continuous access phase. Finally, animals of both lines consumed significantly more water when housed with access to running wheels

relative to animals housed with locked wheels, but there were no significant effects of running-wheel access on either ethanol intake or ethanol preference (Fig. 6).

Experiment 3

Materials and Methods

Animals, apparatus and procedures—Male WSP-2 and WSR-2 mice (N = 8 and 10, respectively) were obtained from the breeding facilities at the Oregon Health and Science University at about 8 weeks of age and upon arrival were placed individually into commercial running-wheel cages. All other details were as described above for Experiment 2. The design and analysis of Experiment 3 was identical to that described above for Experiment 2.

Results

Fig. 7 shows mean weekly ethanol intake across the entire experiment, while Fig. 8 shows ethanol intake, ethanol preference, and water intake for each of the five 6-week experimental phases. Ethanol intake was nearly identical in the two breeding lines throughout the experiment, and was about 30% higher under IA1 conditions relative to initial continuous-access conditions (Figs. 7, 8). Ethanol intake then dropped below initial levels during the second continuous-access phase, and escalated again during IA3 conditions. Notably, unlike Experiments 1 and 2, WSP and WSR mice showed persistent increases in ethanol intake during the final continuous-access phase. ANOVA revealed that ethanol intake differed across experimental phases ($F(4,72) = 45.996$, $p < 0.001$), but failed to detect any significant main effects or interactions involving either breeding line or running-wheel access. Pairwise comparisons between experimental phases showed that ethanol intake was lower during the second than either the first or the third continuous-access phase, and that intake under IA1 access was higher than that seen during any of the three phases of continuous ethanol access. No difference was detected between IA1 and IA3 conditions, or between IA3 conditions and the subsequent continuous-access phase.

Analysis of ethanol preference revealed main effects of experimental phase ($F(4,72) = 30.050$, $p < 0.001$), while pairwise comparisons indicated that ethanol preference varied across experimental phases in a manner very similar to that seen for ethanol intake (Fig. 8). Thus, ethanol preference was significantly different between all pairs of phases other than between IA1 and IA3 and between IA1 and the final continuous-access phase. Analysis of water intake also revealed a main effect of experimental phase ($F(4,72) = 2.916$, $p = 0.027$), as well as a phase by breeding line interaction. Despite this interaction, however, pairwise tests showed no significant differences in water intake between the two breeding lines for any of the 5 experimental phases. Pairwise comparison of water intake between experimental phases showed that water intake differed significantly between the second continuous-access phase and both intermittent-access phases, as well as the third continuous-access phase.

While there were no interactions between running-wheel access and either experimental phase or breeding line, animals with access to running wheels showed significantly lower ethanol preference ($F(4,72) = 12.204$, $p = 0.003$) and significantly higher water intake ($F(1,18) = 5.969$, $p = 0.025$) than those with locked wheels (Fig. 9).

Discussion

Increased ethanol intake following a period of ethanol deprivation (i.e., the ADE) has been proposed to provide an animal model for the excessive drinking that frequently accompanies relapse in abstinent alcoholics (Sanchis-Segura and Spanagel, 2006). In addition, repeated

cycles of ethanol access and deprivation (i.e., RADE and EOD procedures) often result in a progressive acceleration of ethanol intake that may reflect neuroadaptive processes underlying the transition from alcohol abuse to alcohol addiction (Breese et al., 2005; Sanchis-Segura and Spanagel, 2006; Crabbe et al., 2011). Nevertheless, neither the mechanisms underlying the escalation of drinking under intermittent ethanol access nor the exact relevance of such phenomena for the clinical course of alcohol use disorders are known. In the present experiments, we examined the generality of the intermittency effect across genotypes, and tested the specific hypotheses that a genetic propensity for binge-like drinking or withdrawal seizure susceptibility would alter the escalation of drinking in response to intermittent ethanol access.

Previous experiments have shown that intermittent 24-hour ethanol access increases daily ethanol intake in rats and mice, whether ethanol is made available every other day, 3 days per week (IA3), or 1 day per week (IA1). Since these prior studies only tested the B6 mouse strain, the present experiments examined voluntary ethanol intake under continuous, IA1 and IA3 access conditions in a variety of mouse genotypes, including two inbred strains (B6 and C3H), three selectively-bred lines (HDID-1, WSP-2 and WSR-2), and a genetically heterogeneous 8-way cross (HS/Npt). Taken together, the results of the present experiments indicate that intermittent ethanol access results in escalated ethanol intake and ethanol preference in diverse mouse genotypes. Robust effects were seen in both inbred strains, despite their dramatically different ethanol preference and ethanol withdrawal phenotypes. Further, neither selective breeding for binge-like drinking in the DID test nor selective breeding for sensitivity to ethanol withdrawal produced any discernible effect on the response to intermittent ethanol access. Thus, the effects of intermittent ethanol access are broadly generalizable across genetic background. Further, the effects of intermittent ethanol access were not altered by either photoperiod or running-wheel access, two manipulations that have been shown previously to modulate free-choice ethanol drinking, indicating that this effect may also be generalized across environmental conditions as well.

Nevertheless, the magnitude of the intermittency effect did vary across the various genotypes tested in this study. The approximate doubling of intake in B6 mice was very similar to that observed previously by Melendez et al. (2006; 2011) using similar access schedules but a higher ethanol concentration (15% vs. 10% in the present study). In contrast, the most robust effect was seen in C3H inbred mice, which showed an approximate 4-fold increase in ethanol intake, while the least robust effects were seen in WSP-2 and WSR-2 mice, which displayed an approximate 30–50% increase in ethanol intake under intermittent ethanol access. The more robust effect in C3H relative to B6 mice seen in Experiment 1 is consistent with prior indications that the effects of intermittency may be negatively correlated with baseline ethanol intake (Khisti et al., 2006; Simms et al., 2008). On the other hand, HDID-1 and HS/Npt mice showed much more robust effects than did WSP-2 and WSR-2 mice, despite the fact that these four lines displayed very similar ethanol intake under continuous-access conditions. Since these lines are all derived from 8-way inbred-strain crosses (HS/Npt and HS/Ibg), and since 6 of the 8 strains used to create the two crosses were identical, it is perhaps not surprising that these lines all display similar, moderate levels of ethanol preference drinking. In contrast, however, it does seem surprising that the two HS/Ibg-derived lines (WSP-2 and WSR-2) lines differ so dramatically from the HS/Npt and HS/Npt-derived HDID-1 lines in their response to intermittent access. Taken together, therefore, our results confirm previous observations indicating that various ethanol-related phenotypes show only loose genetic coupling in selective breeding experiments. In addition, these results indicate phenotypic or genetic mechanisms beyond those controlling ethanol preference drinking, binge-like DID drinking, and ethanol withdrawal sensitivity must modulate the magnitude of the intermittency effect.

Previous studies using ADE and intermittent access procedures generally report only temporary persistence of above-baseline levels of ethanol intake when animals are returned to continuous-access conditions. Thus, under traditional ADE procedures with multi-day continuous-access drinking periods, rats and mice typically return to baseline levels within a few days, even following multiple deprivations (e.g., Heyser et al., 1997; Rodd-Henricks et al., 2000a, b; Spanagel and Holter, 1999; Khisti et al., 2006). For example, when B6 mice were returned to continuous access following 7–10 cycles of either 1-day per week or EOD ethanol access, intake returned to baseline levels within about a week (Melendez et al., 2006; Melendez, 2011). In the present experiments, we performed weekly measurements of ethanol intake, so it was not possible to track the day-by-day changes that may have occurred when animals were returned to continuous access. Nevertheless, all mice all returned to baseline levels (or below) with 1–2 weeks following IA1. Further, all lines other than C3H showed significant reductions in ethanol intake in the second relative to the first continuous access epoch. This unexpected finding may be analogous to the “negative alcohol deprivation effect” noted previously in both rats (Sinclair and Li, 1989) and mice (Melendez et al., 2006) following extended periods of ethanol deprivation.

Following IA3, HDID-1 and HS/Npt mice showed a rapid return to typically levels of continuous-access drinking, as they had following IA1. In contrast however, WSP-2 and WSR-2 mice showed persistent elevations in ethanol intake throughout the 6 weeks of continuous access following IA3 conditions. Because all animals were exposed first to IA1 and then to IA3, we do not know whether this persistence is due to IA3 exposure per se, or simply to the more extended history of intermittent drinking that had elapsed by this phase of the experiment. In either case, however, these results raise the possibility that a history of intermittent ethanol drinking may result in a long-lasting escalation of drinking under free-choice conditions, at least in some genotypes. If this finding could be replicated, it would strengthen the potential link between the ADE as studied in animals and the hypothesized role of repeated episodes of relapse and remission in promoting the transition to addiction in human drinkers.

Previous studies showed that variations in photoperiod can modulate ethanol intake, both in reproductively photoperiodic hamsters (Geller and Hartmann, 1977) and in reproductively non-photoperiodic rats (Geller, 1971; Goodwin et al., 1999) and mice (Millard and Dole, 1983; Rosenwasser et al., 2010). In contrast, the present experiment detected neither a significant main effect of photoperiod nor any significant photoperiod interactions with either strain or ethanol access conditions. Nevertheless, an exploratory post-hoc analysis showed that C3H mice tended to drink more ethanol under long relative to short photoperiod conditions, potentially related to previous reports of increased depression- and anxiety-like behavior under long photoperiods in this strain (Kopp et al., 1999; Becker et al., 2010). Most importantly for the present study, however, we saw no suggestion that photoperiod can modulate the escalation of ethanol intake under intermittent access to ethanol.

Introduction of a running-wheel has been reported to decrease ethanol intake and/or ethanol preference in rats (McMillan et al., 1995), mice (Ozburn et al., 2008; Ehringer et al., 2009) and hamsters (Hammer et al., 2010). Nevertheless, careful analysis of these studies indicates that reduced ethanol preference in the presence of a running wheel is often due mainly to increased water intake, rather than to reduced ethanol intake. Similarly, in this study access to running wheels resulted robustly increased water intake in all four lines (HDID-1, HS/Npt, WSP-2, WSR-2), while only WSP-2 and WSR-2 mice exhibited significant reductions in ethanol preference, and none of the four lines showed significant effects on ethanol intake. Most importantly, however, we saw no evidence that running-wheel access interacts with ethanol access conditions to modulate ethanol drinking.

In conclusion, we have shown that intermittent ethanol access increases ethanol intake in diverse genotypes and across varying environmental conditions. While the magnitude of this response may depend in part on baseline free-choice ethanol intake, we also observed divergent responses to intermittency in breeding lines with similar levels of ethanol preference. Further, the genetic bases underlying variations in responsiveness to intermittent access appear to be largely distinct from those underlying variations in binge-like drinking and withdrawal sensitivity.

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Figure 1.

Mean ($\pm$ SEM) daily ethanol intake assessed at weekly intervals in C57BL/6J and C3H/HeJ mice ($N = 15$ per strain). “CON”: continuous availability of 10% v/v ethanol in free choice with water. “IA1”: intermittent availability of 10% ethanol in free choice with water one day per week; animals were offered 2 water bottles on the other days.

Figure 2.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in C57BL/6J and C3H/HeJ mice, collapsed into three consecutive 6-week epochs based on ethanol-access conditions. Ethanol was available either continuously (7 days per week) or intermittently (1 day per week).

Figure 3.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in C57BL/6J and C3H/HeJ mice, expressed as a function of photoperiod, and collapsed across ethanol-access conditions. “Long” photoperiods comprised 18 hours of light alternating with 6 hours of darkness (LD 18:6). “Short” photoperiods comprised 6 hours of light alternating with 18 hours of darkness (LD 6:18).

Figure 4.

Mean ($\pm$ SEM) daily ethanol intake assessed at weekly intervals in HDID-1 (N=15) and HS/Npt (N=14) mice. "CON": continuous availability of 10% v/v ethanol in free choice with water. "IA1": intermittent availability of 10% ethanol in free choice with water one day per week; animals were offered 2 water bottles on the other days. "IA3": intermittent availability of 10% ethanol in free choice with water three days per week; animals were offered 2 water bottles on the other days.

Figure 5.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in HDID-1 and HS/Npt mice, collapsed into five consecutive 6-week epochs based on ethanol-access conditions. Ethanol was available either continuously (7 days per week) or intermittently (1 day per week or 3 days per week).

Figure 6.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in HDID-1 and HS/Npt mice, expressed as a function of running-wheel access conditions, and collapsed across ethanol-access conditions. “Free Wheel”: animals had continuous access to running-wheels. “Locked Wheel”: animals lived in identical cages with mechanically locked running wheels.

Figure 7.

Mean ($\pm$ SEM) daily ethanol intake assessed at weekly intervals in WSP-2 (N=8) and WSR-2 (N=10) mice. "CON": continuous availability of 10% v/v ethanol in free choice with water. "IA1": intermittent availability of 10% ethanol in free choice with water one day per week; animals were offered 2 water bottles on the other days. "IA3": intermittent availability of 10% ethanol in free choice with water three days per week; animals were offered 2 water bottles on the other days.

Figure 8.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in WSP-2 and WSR-2 mice, collapsed into five consecutive 6-week epochs based on ethanol-access conditions. Ethanol was available either continuously (7 days per week) or intermittently (1 day per week or 3 days per week).

Figure 9.

Mean ($\pm$ SEM) daily ethanol intake (top panel), water intake (bottom panel) and ethanol preference (middle panel) in WSP-2 and WSR-2 mice, expressed as a function of running-wheel access conditions, and collapsed across ethanol-access conditions. “Free Wheel”: animals had continuous access to running-wheels. “Locked Wheel”: animals lived in identical cages with mechanically locked running wheels.