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ORIGINAL INVESTIGATION

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Voluntary ethanol drinking in C57BL/6J and DBA/2J mice before and after sensitization to the locomotor stimulant effects of ethanol

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Abstract *Rationale:* Drug-induced sensitization has been associated with enhanced drug self-administration and may contribute to drug addiction. *Objectives:* We investigated the possible association between sensitization to the locomotor stimulant effects of ethanol (EtOH) and voluntary EtOH consumption. *Methods:* Mice of the EtOH-avoiding DBA/2J (D2) and EtOH-preferring C57BL/6J (B6) inbred strains were offered the choice of an EtOH solution versus tap water (EtOH-experienced) or just water (Na), and voluntary consumption was measured. Mice from each condition then received repeated EtOH or saline injections, and locomotor responses were measured. Subsequently, all mice were offered the choice of EtOH versus water, and voluntary consumption was again measured. A subsequent study examined relative susceptibility of D2 and B6 mice to EtOH-induced locomotor sensitization. *Results:* Voluntary EtOH consumption induced locomotor sensitization to an EtOH challenge in B6 mice. D2 mice consumed little EtOH, but developed sensitization with repeated EtOH treatments as expected. EtOH consumption was not altered in EtOH-sensitized D2 mice. Unexpectedly, B6 mice developed significant sensitization, and following sensitization, the EtOH-experienced EtOH-sensitized group consumed more EtOH than their EtOH-experienced saline-treated (non-sensitized) counterparts. In an independent study, B6 mice required between three and five EtOH injections to express sensitization, whereas for D2 mice, between one and three EtOH exposures were sufficient. *Conclusions:* Development of sensitization to the locomotor stimulant effects of EtOH may be associated with increased EtOH consumption in mice with high initial avidity for EtOH. In the same mice, voluntary EtOH consumption can also produce behavioral sensitization to the effects of EtOH.

Keywords Ethanol · Alcohol · Sensitization · Reinforcement · Self-administration · C57BL/6J mice · DBA/2J mice

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Introduction

Behavioral stimulation is a common response to many drugs of abuse (Wise and Bozarth 1987). Repeated drug administration may lead to an enhancement of this stimulant response, a process termed behavioral sensitization. Drug-induced sensitization has been hypothesized to reflect neural adaptations important to the development of drug addiction (Wise and Bozarth 1987; Robinson and Berridge 1993). Ethanol (EtOH) has also been shown to induce behavioral sensitization with repeated administration (Masur and Boerngen 1980; Masur et al. 1986; Phillips et al. 1995), possibly accompanied by neuroadaptations that contribute to uncontrolled drinking (Hunt and Lands 1992), and to the development of EtOH abuse and addiction (Schmidt et al. 2000).

Many studies agree that pre-exposure to drugs of abuse is associated with enhancement, or sensitization, of behaviors thought to reflect drug reinforcement (Lett 1989; Horger et al. 1990, 1992; Schenk et al. 1993; Valadez and Schenk 1994; Shippenberg and Heidbreder 1995; Shippenberg et al. 1996; De Vries et al. 1998; Mendrek et al. 1998; Pierre and Vezina 1998; Lorrain et al. 2000). Studies examining this relationship for EtOH show contradictory results. Using voluntary EtOH consumption as an index of EtOH reinforcement (McBride and Li 1998), a pre-exposure period of forced EtOH con-

sumption has been shown to increase (Kampov-Polevoy et al. 2000), decrease (Le Pen et al. 1998), or have no effect (Ufer et al. 1999) on subsequent voluntary EtOH consumption in rats. On the other hand, pre-exposure to drugs of abuse other than EtOH has been consistently associated with increased EtOH consumption, for example following injections of escalating doses of amphetamine (Fahlke et al. 1994) or continuous amphetamine exposure via pellets (Potthoff and Ellison 1982; Potthoff et al. 1983; Levy and Ellison 1985), following repeated cocaine (McMillan and Snodgrass 1991) or morphine injections (Hubbell et al. 1986; Volpicelli et al. 1991), following sensitization to the stimulant effects of nicotine (Blomqvist et al. 1996), and during nicotine administration (Potthoff et al. 1983; Smith et al. 1999). Only one of these studies measured and reported data on drug-induced behavioral sensitization per se (Blomqvist et al. 1996).

Data collected in independent studies of voluntary EtOH consumption (Phillips et al. 1994a) and EtOH-induced sensitization (Phillips et al. 1995) in C57BL/6J (B6) × DBA/2J (D2) BXD recombinant inbred mouse strains indicated that strains most susceptible to the locomotor sensitizing effects of EtOH were the ones that voluntarily consumed the least EtOH (Phillips et al. 1995). This negative genetic correlation is consistent with the greater sensitivity to EtOH-induced sensitization seen in the EtOH-avoiding D2 strain relative to the EtOH-preferring B6 strain (Cunningham et al. 1992; Phillips et al. 1994b, 1996), and indicates some overlap in the genetic determinants of EtOH-induced sensitization and voluntary EtOH consumption.

Since the studies described here were initiated, we are aware of only two reports in which EtOH sensitization and voluntary EtOH drinking were assessed in the same animals. One suggested a positive association between EtOH-induced sensitization and EtOH drinking using D2 and B6 mice (Camarini et al. 2000), and the other (our own) suggested a negative association between these phenotypes in genetically heterogeneous mice (Lessov and Phillips 1998). Recently, Grahame and colleagues (2000a) showed that EtOH-na mice selected for high alcohol preference developed sensitization to the locomotor stimulant effects of EtOH, whereas mice selected for low alcohol preference did not, suggesting a positive genetic association between these phenotypes.

The present experiments were designed to determine whether mice sensitized to the locomotor stimulant effects of EtOH would exhibit a change in subsequent voluntary EtOH consumption, and whether voluntary EtOH drinking itself would induce sensitization. The high EtOH-consuming, low-sensitizing B6 mice, and the low EtOH-consuming, high-sensitizing D2 mice were used in these studies. Through the differential EtOH sensitization and self-administration profiles of these strains, we sought to examine the relationship between EtOH-induced sensitization and EtOH consumption.

Materials and methods

Three experiments were conducted. In experiments 1 and 2, the potential association between sensitization to the effects of EtOH and voluntary EtOH consumption was investigated in B6 and D2 mice. Experiment 3 was designed to compare the time course and magnitude of EtOH-induced sensitization between these inbred strains.

Subjects

Female (experiments 1 and 2) or male (experiment 3) mice from the B6 and D2 inbred strains were used. For experiments 1 and 2, female mice were chosen to facilitate comparison with prior data from this laboratory; female rats have also been characterized as more susceptible to drug-induced sensitization (Robinson 1984; Cailhol and Mormède 1999). For experiment 3, male mice were chosen in part because pilot data indicated that the sex difference in sensitivity to sensitization did not appear to apply to EtOH. Mice were obtained from the Jackson Laboratory (Bar Harbor, Me., USA) at least 1 week prior to utilization. For experiments 1 and 2, mice were acclimated to a reverse 12:12 h light:dark cycle (lights off at 9 or 10 a.m.) for a minimum of 2 weeks to permit collection of drinking data at several time points during the dark phase. For experiment 3, mice were kept on our usual 12:12 h light cycle (lights on at 9 a.m.). Animals had food and water freely available, except during behavioral testing. At the beginning of experiments 1 and 2, mice were 74 and 66 days old, respectively. At the beginning of experiment 3, mice were 52–54 days old. All experiments were performed in accordance with the Institutional Animal Care and Use Committee and National Institutes of Health guidelines for the care and use of laboratory animals.

Drugs

For injections, 100% EtOH (Pharmco Products, Brookfield, Conn., USA) was diluted in saline (0.9% NaCl; Baxter Healthcare, Deerfield, Ill., USA) for a final solution of 20% v/v. Saline was used for control injections. All injections were given intraperitoneally. As a drinking fluid, EtOH was mixed with tap water for final solution concentrations of 3%, 6%, or 10% EtOH v/v.

Apparatus

Mice were tested in automated locomotor activity monitors (40×40×30 cm; Accuscan, Columbus, Ohio, USA) housed in sound-attenuating chambers. Lights and fans were mounted in the middle or upper corner, respectively, of each chamber rear wall. Lights were on or off during behavioral testing, consistent with the particular study's light cycle (see Subjects or Procedures); fans were on, providing ventilation and a constant background noise. Eight infrared beams were mounted 2 cm above the test chamber floor on two perpendicular panels. Eight detectors were mounted on opposing panels. As mice moved about the chamber floor, infrared beam interruptions were automatically recorded as activity counts. Data were automatically translated to horizontal distance traveled (centimeters), generally interpreted as walking or running behavior, and this is the reported measure of locomotor activity.

Procedures

Experiments 1 and 2: EtOH-induced sensitization and EtOH consumption in D2 and B6 mice

Experiment 1 determined the effects of voluntary EtOH consumption on sensitivity to the locomotor stimulant effects of EtOH, and

the effects of repeated EtOH administration on subsequent voluntary EtOH consumption in B6 and D2 mice. Experiment 2 extended the most significant findings from experiment 1 using only B6 mice. There were 6–8 mice per group in experiment 1, and 12 mice per group in experiment 2. Each experiment consisted of three phases: (1) presensitization drinking, (2) EtOH-induced sensitization, and (3) postsensitization drinking.

Presensitization drinking. Mice were single housed and presented with two water-containing 25-ml calibrated tubes adapted with drinking spouts for 4 days. For the subsequent 12 (experiment 1) or 8 (experiment 2) consecutive days, half of the animals had one of their water tubes replaced with a tube containing EtOH (EtOH-experienced). In experiment 1, EtOH was presented in ascending concentrations of 3%, 6%, or 10% EtOH in an attempt to fully evaluate EtOH drinking in the normally EtOH-avoiding D2 mice. Each concentration was available 24 h/day for 4-day periods. In experiment 2, B6 mice were immediately presented with 10% EtOH, known to be their preferred concentration. The second half of the animals were presented with water only (Na). Water and EtOH consumption volume was recorded immediately before lights off (9 a.m. for experiment 1; 10 a.m. for experiment 2), in addition to 3 (experiment 1) and 6 h into the dark cycle (experiments 1 and 2). For data presentation and analyses, EtOH consumption was expressed as grams EtOH consumed in 24 h per kilogram body weight. Drinking volumes were adjusted according to leakage/evaporation determined from tubes placed on empty cages. Body weight was measured every 4 days. Drinking tube positions were alternated every 48 h to negate any arbitrary side preference. Averages were calculated based on data collected on days 2 and 4 of every 4-day access period as the most stable estimate of EtOH consumption (Phillips et al. 1994a). Water and EtOH drinking tubes were replaced with clean ones every 8 and 4 days, respectively.

Final water and EtOH consumption volumes were recorded in the morning following the 4th day of the last 4-day period. Drinking tubes were replaced with standard water bottles. Equal numbers of mice from the Na and EtOH-experienced conditions were assigned to saline (Repeated Saline) or EtOH (Repeated EtOH) treatment groups, resulting in four treatment groups: Na-Repeated Saline, Na-Repeated EtOH, EtOH-experienced Repeated Saline, and EtOH-experienced Repeated EtOH.

EtOH-induced sensitization. This phase was initiated 24 h later. On activity test days, mice were moved to the testing room immediately before lights off and allowed to acclimate to the dark testing environment for at least 90 min. On days 1 and 2, all mice were injected with saline and immediately placed in the center of an activity monitor for a 10-min test to permit habituation to the testing apparatus (Day 1) and to obtain baseline activity data (Day 2). On Day 3, Na- and EtOH-experienced Repeated Saline groups were tested following saline, while Na- and EtOH-experienced Repeated EtOH groups were tested following 2 g/kg EtOH to obtain acute EtOH stimulant response data. For the subsequent 10 consecutive days (days 4–13), the two Repeated Saline groups received daily saline injections and the two Repeated EtOH groups received daily 2.5 g/kg EtOH injections. Mice were returned to their home cages following injection. No activity testing took place. Twenty-four hours following the final daily injection, the Na- and EtOH-experienced Repeated Saline groups were tested following saline, while the Na- and EtOH-experienced Repeated EtOH groups were tested following 2 g/kg EtOH injections. Sensitization to the effects of EtOH was assessed as a within-group measure by comparing the final EtOH response of each Repeated EtOH group on Day 14 to their acute EtOH response on Day 3.

Postsensitization drinking. This phase was initiated 24 h later. All animals (instead of half) were offered a choice between water and ascending concentrations of 3%, 6%, and 10% EtOH for experiment 1, and only 10% EtOH for experiment 2, 24 h/day, for the duration of 12 or 8 days, respectively.

Experiment 3: development of EtOH-induced sensitization in D2 and B6 male mice

In experiments 1 and 2, B6 mice showed unexpected development of sensitization to the stimulant effects of EtOH. This experiment was specifically designed to extend those results by determining the number of EtOH exposures required to induce sensitization in B6 mice. The time course and magnitude for the development of sensitization to EtOH was compared to that of D2 mice.

B6 and D2 mice were randomized into Repeated Saline and Repeated EtOH groups. EtOH-induced sensitization was evaluated in a between-group design in which paired groups of Repeated Saline and Repeated EtOH mice were evaluated following 1, 3, 5, or 10 saline or EtOH exposures. On Days 1 and 2, all mice were tested following saline injections. On Day 3, Repeated Saline and Repeated EtOH groups received saline or 2.5 g/kg EtOH injections, respectively, and were returned to their home cages. On Day 4, the pair of Repeated Saline and Repeated EtOH groups designated to receive one EtOH pre-exposure was tested following 2 g/kg EtOH, while the remaining groups received their second consecutive treatment pre-exposure of saline or 2.5 g/kg EtOH. This pattern was followed until independent 3, 5, and 10 pre-exposure pairs of groups received the designated number of consecutive saline or 2.5 g/kg EtOH treatments and were tested following 2 g/kg EtOH 24 h after their final pre-exposure. Activity data were collected for 15 min.

Immediately following activity testing, a 20-µl tail blood sample was collected from each mouse for blood ethanol concentration (BEC) determination. Blood was expelled into a microcentrifuge tube containing 50 µl 5% $ZnSO_4$ and was mixed with 50 µl $Ba(OH)_2$ and 300 µl of ddH_2O. Tube contents were spun at 12,000 rpm for 5 min (Beckman Microfuge, San Jose, Calif., USA). The supernatant was collected and analyzed by a gas chromatograph (Hewlett Packard, Palo Alto, Calif., USA) with a flame ionization detector.

Statistical analyses

Experiments 1 and 2: EtOH-induced sensitization and EtOH consumption in D2 and B6 mice

All data were analyzed separately for each of the D2 and B6 strains. Presensitization drinking levels were compared using one-way ANOVA with Group (Repeated Saline vs Repeated EtOH) as the independent variable, and 10% EtOH consumption (grams/kilogram per day) as the dependent variable. After analyzing data for all EtOH concentrations and for all time points, 24-h 10% EtOH consumption was chosen as a representative measure of EtOH drinking behavior. Data interpretation was not altered by considering the lower EtOH concentrations for D2 mice.

EtOH sensitization was first analyzed using Group (Repeated Saline vs Repeated EtOH) × Drinking Experience (Na vs EtOH-experienced) × Test Day (Days 1, 2, 3, and 14) ANOVAs, with total 10-min horizontal distance traveled (centimeters) as the dependent variable. The development of within-group sensitization for each of the Na- and EtOH-experienced Repeated EtOH groups was evaluated using one-way ANOVAs with Test Day as the independent variable. The effect of prior drinking experience on subsequent response to acute EtOH injection was evaluated by comparing the Na-Repeated EtOH and EtOH-Repeated EtOH groups using Drinking Experience × Test Day ANOVAs and was also assessed based on the one-way ANOVAs described above.

Postsensitization EtOH consumption (grams/kilogram per day) was analyzed using Group × Drinking Experience ANOVAs. Presensitization vs postsensitization drinking levels for the EtOH-experienced groups were compared using Group (Repeated Saline vs Repeated EtOH) × Time (pre- vs postsensitization) ANOVAs.

Experiment 3: development of EtOH-induced sensitization in D2 and B6 male mice

Data were analyzed using Strain (D2 vs B6) × Group (Repeated Saline vs Repeated EtOH) × Treatment Number (1, 3, 5, or 10) ANOVA. The number of EtOH treatments necessary to induce sensitization was determined in two ways, separately for each strain, with a Group × Treatment Number ANOVA, and with one-way ANOVAs for each Treatment Number with Group as the independent variable.

For all experiments, where appropriate, significant main and interaction effects were followed up with further ANOVAs, simple main effects analyses, and/or Newman-Keuls post hoc multiple comparisons tests.

Results

Experiment 1: EtOH-induced sensitization and EtOH consumption in D2 and B6 mice

Presensitization drinking in D2 and B6 mice

D2 mice consumed small amounts of EtOH (Fig. 1A), whereas B6 mice consumed large amounts of EtOH (Fig. 2A). There were no significant differences in presensitization drinking levels between the treatment groups designated for subsequent repeated saline or repeated EtOH treatment for either strain.

EtOH-induced sensitization in D2 and B6 mice

EtOH administration significantly affected locomotor response across test days for both D2 (Fig. 1B) and B6 (Fig. 2B) mice [significant Group × Test Day interaction effects; $F(3,69)=245.2$ for D2 mice and $F(3,66)=24.0$ for B6 mice, both $P<0.001$]. Follow-up analyses (Group × Test Day ANOVAs) performed separately for each of the Na and EtOH-experienced conditions showed that for D2 mice, Repeated Saline and Repeated EtOH groups had differential patterns of response across test days [significant Group × Test Day interaction effects; $F(3,39)=107.1$ for the Na condition and $F(3,30)=171.2$ for the EtOH-experienced condition, both $P<0.001$]. This was due to significant EtOH-induced stimulation of both Repeated EtOH groups on Days 3 and 14 relative to the saline response of the respective Repeated Saline controls on those days (simple main effects; all four $P<0.001$). On Day 3, the Na- and EtOH-experienced Repeated EtOH groups showed similar degrees of EtOH-induced stimulation, indicating lack of sensitization to the effects of EtOH as a result of EtOH drinking experience.

Follow-up analyses for B6 mice also showed significant differential patterns of response between the Repeated Saline and Repeated EtOH groups for each of the Na and EtOH-experienced conditions across test days [significant Group × Test Day interaction effects; $F(3,36)=7.4$ for the Na condition, $F(3,30)=18.1$, for the EtOH-experienced condition, both $P<0.001$]. There was

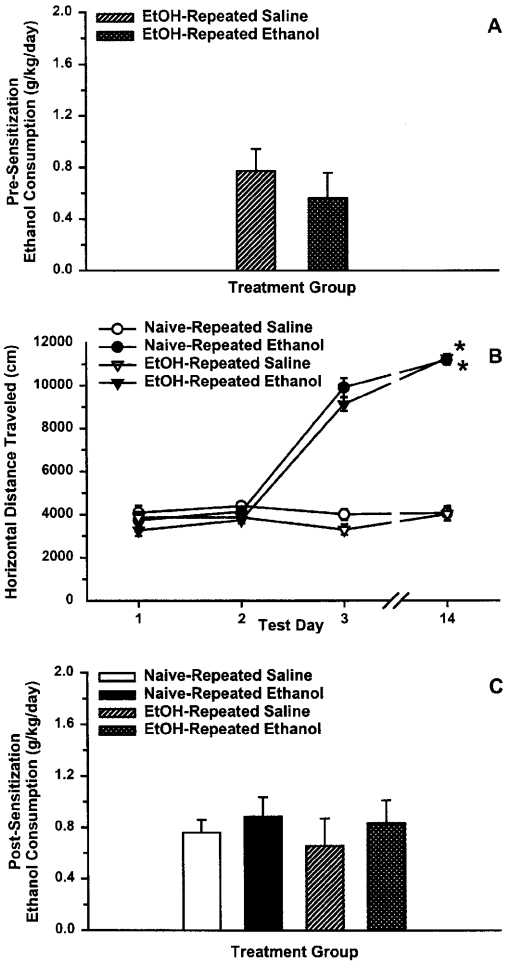

Fig. 1A–C Experiment 1. D2 mice 24-h 10%EtOH consumption (g/kg per day) before sensitization (**A**) and after sensitization (**C**), and total 10-min locomotor activity across activity test days 1, 2, 3, and 14 (**B**). **A** Groups with presensitization EtOH drinking experience (EtOH-) subsequently administered saline (Repeated Saline, *diagonal bar*) or EtOH (Repeated EtOH, *hatched bar*) injections. **B** Groups with (EtOH-, *triangles*) or without (Na-, *circles*) presensitization EtOH drinking experience that received saline (Repeated Saline, *clear symbols*) or EtOH (Repeated EtOH, *filled symbols*) injections. **C** Groups with (EtOH-) and without (Na-) presensitization EtOH drinking experience, subsequently administered saline (Repeated Saline, *clear* and *diagonal bars*) or EtOH (Repeated EtOH, *filled and hatched bars*) injections. n=6–8 per group. * Significant enhancement of EtOH response on Day 14 relative to Day 3 for each Repeated EtOH group; both $P<0.05$

significant EtOH-induced stimulation in both Repeated EtOH groups on Day 14 relative to the saline response of the respective Repeated Saline controls on that day (simple main effects; both $P<0.001$). On Day 3, the EtOH-experienced Repeated EtOH group showed significant EtOH-induced stimulation relative to the saline response of the EtOH-experienced Repeated Saline controls (simple main effects; $P<0.001$), whereas no EtOH vs saline response differences were detected between the Na-Repeated EtOH and Na-Repeated Saline groups on this day. In addition, comparison of the two Repeated EtOH groups showed a tendency for higher EtOH-induced

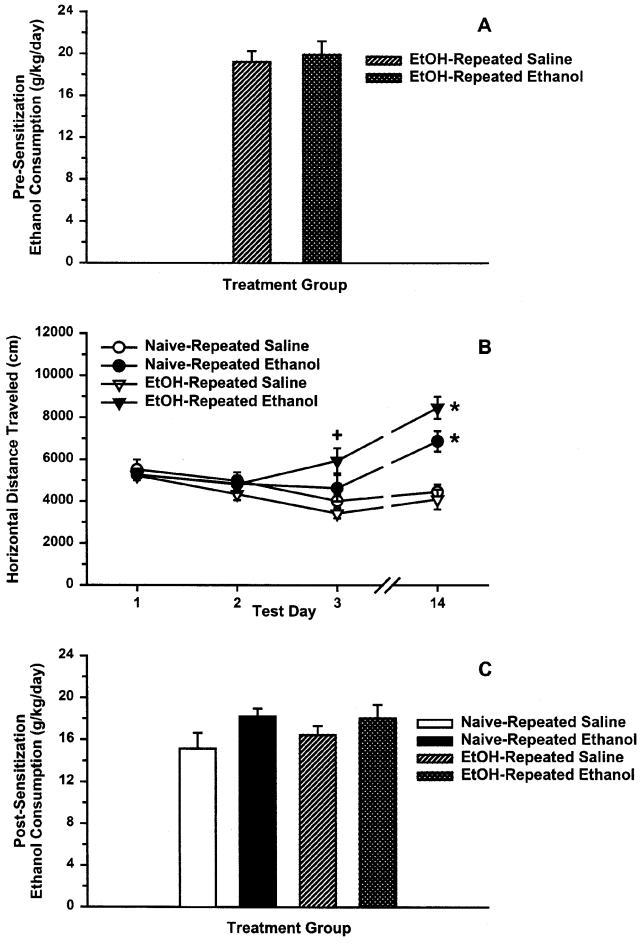

Fig. 2A–C Experiment 1. B6 mice 24-h 10% EtOH consumption (g/kg per day) before sensitization (**A**) and after sensitization (**C**), and total 10-min locomotor activity across activity test days 1, 2, 3, and 14 (**B**). Symbols and procedures are the same as in the legend of Fig. 1. *n*=6–7 per group. * Significant enhancement of EtOH response on Day 14 relative to Day 3 for each Repeated EtOH group; both $P<0.001$. + Significant difference between EtOH-Repeated EtOH and EtOH-Repeated Saline groups on Day 3; $P<0.001$

acute stimulant response on Day 3 in the EtOH-experienced group relative to their Na counterparts [trend for an interaction effect; $F(3,33)=2.7$, $P=0.058$]. These results suggest that prior drinking sensitized B6 mice to the stimulant effects of EtOH.

For both D2 and B6 mice, both Na- and EtOH-experienced Repeated EtOH groups developed significant within-group sensitization to EtOH as evidenced by their enhanced EtOH responses on Day 14 relative to their acute EtOH responses on Day 3 (Newman-Keuls; all $P<0.01$).

Postsensitization drinking in D2 and B6 mice

There were no effects of repeated EtOH administration or prior drinking experience on postsensitization EtOH consumption in D2 mice (Fig. 1C). In contrast, B6 mice (Fig. 2C) showed a trend toward overall greater alcohol consumption in the Repeated EtOH groups relative to their Repeated Saline counterparts ($P=0.058$). However, within-group comparison of pre- vs postsensitization drinking indicated no significant increase in postsensitization EtOH consumption relative to initial consumption levels for either D2 or B6 mice, regardless of sensitization treatment.

Experiment 2: EtOH-induced sensitization and EtOH consumption in B6 mice

Experiment 1 suggested that the experimental manipulations were unlikely to result in significant alterations in EtOH drinking behavior in D2 mice. In addition, experiment 1 resulted in unexpected development of EtOH-induced sensitization in B6 mice (Fig. 2B). The current experiment aimed to replicate the most significant findings from experiment 1 in a larger sample of only B6 mice: (1) the significant EtOH-induced sensitization in B6 mice, (2) the significant enhancement of sensitivity to the acute stimulant effects of EtOH following EtOH drinking, and (3) the nearly significant enhancement of EtOH drinking in mice sensitized to the locomotor stimulant effects of EtOH.

Presensitization drinking in B6 mice

There were no significant differences in drinking levels between the groups designated for subsequent repeated saline or repeated EtOH treatments (Fig. 3A).

EtOH-induced sensitization in B6 mice

Activity data are presented in Fig. 3B. EtOH administration significantly affected locomotor response across test days [significant Group × Test Day interaction effect; $F(3,132)=73.3$, $P<0.001$]. Follow-up analyses (Group × Test Day ANOVAs) performed separately for each of the Na and EtOH-experienced conditions revealed significant group differences between the Repeated Saline and Repeated EtOH groups on both Days 3 and 14 (simple main effects; all four $P<0.01$). On Day 3, the EtOH-experienced Repeated EtOH mice showed a significant acute stimulant response to EtOH relative to the saline response of the EtOH-experienced Repeated Saline group on that day, and relative to their own baseline Day 2 activity levels (one-way ANOVA; $P<0.02$). The Na-Repeated EtOH group also showed significant acute stimulant response to EtOH on Day 3 relative to Na saline-treated controls on that day, but not relative to their own saline baseline activity levels on Day 2. Comparison between the two Repeated EtOH groups indicated similar patterns of locomotor activity across test days. However, there was a significant main effect of Drinking Experience [$F(1,22)=8.3$, $P<0.01$] with overall greater locomotor activity in the EtOH-experienced relative to

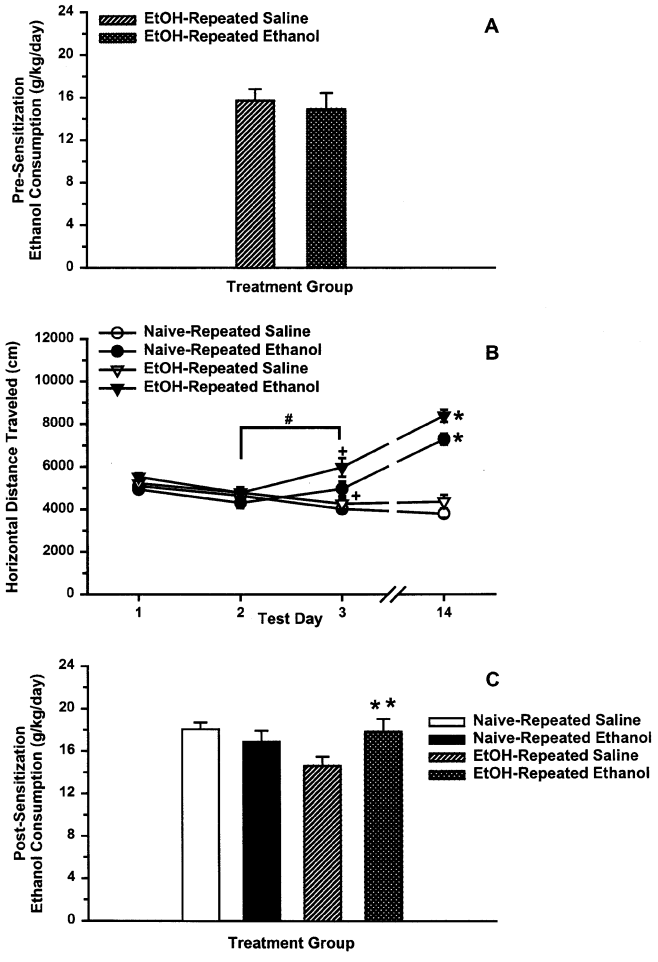

Fig. 3A–C Experiment 2. B6 mice 24-h 10% EtOH consumption (g/kg per day) before sensitization (**A**) and after sensitization (**C**), and total 10-min locomotor activity across activity test days 1, 2, 3, and 14 (**B**). Symbols and procedures are the same as in the legend of Fig. 1. n=12 per group. * Significant enhancement of EtOH response on Day 14 relative to Day 3 for each Repeated EtOH group; both P<0.001. + Significant difference between EtOH-Repeated Saline and EtOH-Repeated EtOH groups, and between Na-Repeated Saline and Na-Repeated EtOH groups on Day 3; both P<0.01. # Significant stimulant acute EtOH response of the EtOH-Repeated EtOH group (*black triangles*) on Day 3 relative to their saline response on Day 2; P<0.02. ** Greater postsensitization EtOH consumption of the EtOH-Repeated EtOH group relative to the EtOH-Repeated Saline group, and relative to their own presensitization consumption levels; both P<0.05

the Na group. It is clear from Fig. 3B that the source of this difference comes mainly from the EtOH responses on Days 3 and 14. These data suggest that sensitization to the locomotor stimulant effects of EtOH was induced by prior EtOH drinking. Significant within-group sensitization was present in both Na- and EtOH-experienced Repeated EtOH groups (both P<0.001).

Postsensitization drinking in B6 mice

The effect of repeated EtOH injections on postsensitization EtOH consumption varied as a function of prior drinking experience [Fig. 3C; Group × Drinking Experience interaction effect; $F(1,44)=5.3$, P<0.05]. The EtOH-experienced Repeated Saline group consumed significantly less EtOH relative to both EtOH-experienced Repeated EtOH (P<0.05), and Na-Repeated Saline (P<0.05) groups. It appears that for B6 mice with prior drinking experience, a sensitizing EtOH regimen enhanced EtOH consumption relative to saline-treated controls. Within-group pre- vs postsensitization comparisons strengthened this conclusion [significant Group × Time interaction effect; $F(1,22)=5.2$, P<0.05] by showing increased postsensitization EtOH consumption in the Repeated EtOH group (P<0.05), but not in the Repeated Saline group (P=0.37).

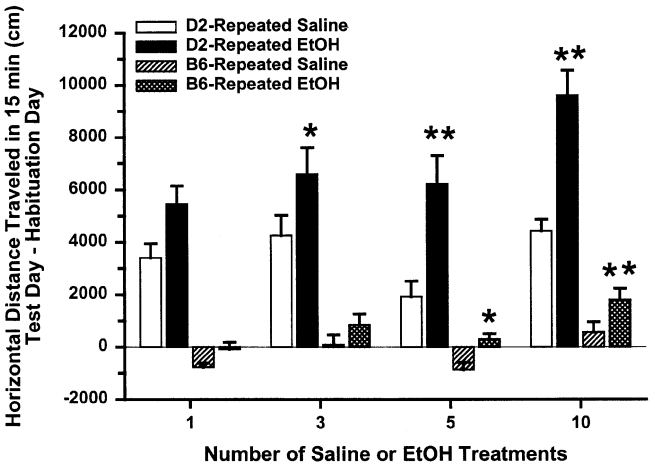

Fig. 4 Experiment 3. Total corrected (test day – baseline) 15-min locomotor activity scores for Repeated Saline (*clear* and *diagonal bars*) and Repeated EtOH (*filled* and *hatched bars*) groups, following 1, 3, 5, or 10 saline or EtOH exposures, respectively, for each of the D2 and B6 strains; n=9–10 per group. * P<0.05 and **P<0.01 between Repeated EtOH and Repeated Saline groups of each strain

Experiment 3: development of EtOH-induced sensitization in D2 and B6 male mice

D2 and B6 mice differed in baseline activity, therefore EtOH stimulant scores were corrected by subtracting Day 2 strain mean from each mouse's EtOH day score (EtOH test day – baseline day for the total 15-min activity test). Because in experiments 1 and 2, activity data were collected for 10 min, data from both the first 10- and total 15-min test period were analyzed. Results with regard to the development of sensitization in each strain were similar for the two time periods, thus only the total 15-min data are shown here.

Figure 4 shows greater activity in D2 relative to B6 mice [Strain; $F(1,140)=275.1$, P<0.001], in Repeated EtOH relative to Repeated Saline groups [Group; $F(1,140)=53.5$, P<0.001], and with increasing number of treatments [Treatment Number; $F(1,1400=11.6$, P<0.001]. Within each strain, both EtOH treatment and

greater number of treatments significantly enhanced locomotor activity [main effects of Group and Treatment Number; D2 mice: $F(1,70)=38.45$ for Group and $F(3,70)=5.5$ for Treatment Number, both $P<0.01$; B6 mice: $F(1,70)=17.3$ for Group and $F(3,70)=11.1$ for Treatment Number, both $P<0.001$]. The lack of significant interaction effects for either strain ($P=0.16$ for D2 mice and $P=0.79$ for B6 mice) precluded a conclusion that sensitization magnitude increased with increasing number of EtOH treatments. However, the second set of analyses (one-way ANOVAs) indicated that D2 mice developed nearly significant sensitization after a single EtOH exposure [$F(1,70)=3.3$, $P=0.07$], and significant sensitization following 3, 5, and 10 EtOH treatments [Fs(1,70)=4.5, 15.2, and 20.8, Ps<0.05, 0.001, and 0.0001]. In B6 mice, significant EtOH-induced sensitization was seen following 5 and 10 EtOH treatments [Fs(1,70)=6.0 and 7.6, Ps<0.05 and 0.01).

Blood ethanol concentrations (BECs)

D2 mice had higher BECs than B6 mice [$F(1,144)=28.0$, $P<0.001$; mean ± SEM for D2 mice=2.39±0.04 and for B6 mice=2.18±0.03 mg/ml], and BEC values varied across Treatment Number [$F(3,144)=14.5$, $P<0.001$; mean ± SEM for Treatment Number 1, 3, 5, and 10=2.19±0.04, 2.21±0.05, 2.51±0.05, and 2.22±0.04 mg/ml, respectively]. Follow-up mean comparisons indicated a significant elevation in BECs in mice receiving five treatments vs all others (Newman-Keuls; all $P<0.01$). However, this significant effect arose only as a main effect, and was not dependent upon saline vs EtOH treatment. The absence of a significant effect of EtOH treatment on BECs, and of any significant interaction effects, indicated that repeated EtOH exposure did not alter EtOH metabolism.

Discussion

Following a sensitizing regimen of EtOH administration, EtOH-experienced B6 mice, which also unexpectedly exhibited locomotor sensitization to the effects of EtOH, increased their alcohol drinking relative to saline-treated controls and relative to their own presensitization drinking levels. D2 mice exhibited locomotor sensitization to the effects of EtOH, yet showed no change in EtOH consumption. This suggests that behavioral sensitization to EtOH may not necessarily be associated with greater avidity for EtOH. However, this conclusion must be tempered by the knowledge that the D2 strain is not simply a strain with lower preference for EtOH than B6, but is a strain with extreme avoidance. Much of this avoidance may be associated with non-pharmacological features of EtOH such as taste and odor (Belknap et al. 1977, 1978; Morrow et al. 1993). Thus, even if the pharmacological consequences of voluntary EtOH intake were altered by prior EtOH sensitization, D2 mice might never experience this due to their EtOH avoidance.

The unexpected presence of EtOH-induced sensitization in the B6 mice precluded our initial aim of comparing EtOH consumption between an EtOH-sensitizing vs a non-sensitizing strain. Procedural differences may account for the EtOH-induced sensitization seen in B6 mice in the current study, and lack thereof in previous studies where EtOH was administered to B6 mice every other day (Cunningham et al. 1992; Phillips et al. 1995, 1996) or daily (Phillips et al. 1994b) and mice were repeatedly tested, rather than being tested after only the initial and final EtOH exposures. In addition, in previous studies both the repeated and testing EtOH doses were 2 g/kg, while in the present experiments the repeated EtOH dose was 2.5 g/kg.

It is difficult to explain why significantly enhanced drinking in sensitized B6 mice was seen only for the group that had been allowed to drink EtOH prior to sensitization, and not in the Na group. This was seen only in experiment 2, in which there was clearly greater power to detect a significant difference due to the larger group size; in experiment 1, there was a tendency for both Na and EtOH-experienced sensitized B6 mice to show an increase in EtOH consumption. The change from an ascending series of EtOH concentrations in experiment 1 to offering only 10% EtOH in experiment 2 could have affected the results and could have additionally contributed to the higher presensitization consumption levels of B6 mice in experiment 1. It may be that prior drinking experience, innate EtOH preference, and proclivity toward the development of sensitization to EtOH all interact to ultimately determine the level of EtOH consumption.

B6 mice with prior EtOH drinking experience showed a significant stimulant response to acute EtOH challenge in both experiments 1 and 2, consistent with other data in this strain (Nocjar and Middaugh 1997), and with data in selectively bred high alcohol preferring mice (Grahame et al. 2000b). In experiment 2, Na B6 mice also showed a significant stimulant response to acute EtOH challenge. However, there was a significant effect of prior drinking experience on locomotor activity that appeared to be associated with a larger response to EtOH in EtOH-experienced mice on acute and sensitization test days. Our experiments suggest that sensitization to EtOH challenge can be induced by a period of voluntary EtOH consumption.

EtOH-induced sensitization in the B6 mice was replicated twice in a within-group design (experiments 1 and 2), and was also shown in a between-group design in male B6 mice (experiment 3). Evaluation of the development of sensitization to the effects of EtOH in the D2 and B6 strains showed that a single EtOH exposure was nearly sufficient to induce sensitization in D2 mice, with significant sensitization emerging following three EtOH injections. In B6 mice, a minimum of five EtOH exposures was required to induce sensitization confirming that D2 mice are more prone to the behaviorally sensitizing effects of EtOH relative to B6 mice (Phillips et al. 1994b, 1996). EtOH metabolism is unlikely to account

for the development of EtOH-induced sensitization since there were no differences in blood alcohol concentrations between the EtOH and saline treatment groups.

The previously detected negative genetic correlation between magnitude of EtOH-induced sensitization and voluntary EtOH consumption (Phillips et al. 1995) was not supported by the current results. Here, a different procedure was used to induce sensitization that appears to have been more robust, given that even a strain with low susceptibility to behaviorally expressed sensitization displayed it. The studies in this paper do show that, in an alcohol-preferring genotype, sensitization to the stimulant effects of EtOH can develop as a function of EtOH self-administration and that in the same genotype, EtOH-sensitized mice tend to voluntarily consume more EtOH relative to non-sensitized controls and relative to pre-sensitization drinking levels. Thus, in line with data showing development of sensitization to the effects of EtOH in selectively bred high alcohol preferring mice (Grahame et al. 2000a) and with the literature on other drugs of abuse, our data support the hypothesis that neural adaptations altered by EtOH exposure and measurable as behavioral sensitization may be important in alcohol consumption (Hunt and Lands 1992) and in the etiology of EtOH abuse and addiction (Wise and Bozarth 1987; Robinson and Berridge 1993; Koob and Le Moal 1997; Schmidt et al. 2000). The dopamine D_2 receptor may be a good candidate for such neuroadaptations, as it has been implicated in both the acute locomotor stimulant and sensitizing effects of EtOH (Broadbent et al. 1995; Shen et al. 1995; Cohen et al. 1997; Phillips et al. 1998b; Souza-Formigoni et al. 1999), and in the mediation of alcohol drinking behavior (Phillips et al. 1994a, 1998a, b), and EtOH reinforcement (Risinger et al. 2000).

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