Published in final edited form as:

Eur Neuropsychopharmacol. 2014 June ; 24(6): 856–864. doi:10.1016/j.euroneuro.2014.01.022.

Adolescent intermittent ethanol exposure diminishes anhedonia during ethanol withdrawal in adulthood

Nathalie Boutros, Svetlana Semenova, and Athina Markou*

University of California San Diego, La Jolla, CA

Abstract

Adolescent alcohol use may interfere with neurodevelopment, increasing the likelihood of adult alcohol use disorders (AUDs). We investigated whether adolescent intermittent ethanol (AIE) exposure alters the adult reward response to ethanol. Adolescent rats were administered ethanol once (moderate exposure; Cohort 1) or three times per day (severe exposure; Cohort 2) in a 2 days on/2 days off pattern. In adulthood, subjects responded for electrical stimulation directed at the posterior lateral hypothalamus in a discrete-trials intracranial self-stimulation (ICSS) procedure that provides current-intensity thresholds as a measure of brain reward function. The effects of ethanol administration and withdrawal were assessed. Control rats showed dose-dependent threshold elevations after acute ethanol, indicating reward deficits. A majority of moderately AIE-exposed rats (Cohort 1) showed threshold lowering after ethanol, suggesting ethanol-induced reward enhancement in this sub-set of rats. Rats exposed to severe AIE (Cohort 2) showed no threshold elevation or lowering, suggesting blunted affective ethanol response. Daily ethanol induced threshold elevations 24 h after administration in control but not in either group of AIE-exposed rats, suggesting decreased sensitivity to the negative affective state of ethanol withdrawal. Withdrawal from a 4-day ethanol binge produced robust and enduring threshold elevations in all rats, although threshold elevations were diminished in rats exposed to severe AIE. These results indicate that AIE exposure diminished reward deficits associated with ethanol intoxication and withdrawal and may have increased ethanol-induced reward enhancement in a sub-set of rats. In humans, enhanced ethanol reward accompanied by reduced withdrawal severity may contribute to the development of AUDs.

© 2014 Elsevier B.V. and European College of Neuropsychopharmacology. All rights reserved.

*Corresponding Author: Dr. Athina Markou, Department of Psychiatry, M/C 0603, School of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0603, USA. Tel: +1 858 534 1572. Fax: +1 858 534 9917. amarkou@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Disclosures

AM has received contract research support from Bristol-Myers Squibb, Forest Laboratories and Astra-Zeneca, and honoraria/consulting fees from AbbVie during the past 3 years. The remaining authors report no financial conflicts of interests.

Author Disclosures

NB, SS and AM designed this project. NB performed the experiments and conducted data analyses. NB, SS and AM wrote the manuscript. All authors discussed the results and commented on the manuscript.

Author Disclosures

This work was supported by National Institutes of Health grant U01-AA019970 (Neurobiology of Adolescent Drinking in Adulthood, NADIA project) to AM. The NIH had no further role in the study design, collection, analysis, and interpretation of the data, writing of the report, or decision to submit the article for publication.

Keywords

adolescent; binge drinking ethanol; self-stimulation; Wistar rats

INTRODUCTION

A large proportion of adolescents worldwide engage in regular heavy alcohol use. The proportion of 16 year olds that report having consumed five or more drinks on a single occasion in the last 30 days exceeds 30% in most countries of Europe, with 17% of students reporting that they engage in such binge drinking episodes at least once a week (Hibell et al., 2009). Similarly, nearly a quarter of American 12th grade students report at least one binge drinking episode in the last two weeks (Johnston et al., 2013). This heavy alcohol use is a major public health concern because early alcohol use initiation predicts later alcohol abuse and the development of alcohol use disorders (AUDs) in adulthood (Grant & Dawson, 1997). Adolescent alcohol use may increase adult AUDs by altering the cortical and subcortical brain reward circuits that mature during adolescence (Spear, 2000). High sensitivity to the rewarding effects of alcohol and low sensitivity to the aversive effects of alcohol predict later heavy alcohol use (King et al., 2011; Schuckit, 1994), demonstrating a relationship between alcohol sensitivity and AUDs, but leaving open the question of causality. Altered sensitivity to ethanol may be an inherited phenotype, or may reflect neuroadaptations engendered by alcohol exposure during developmentally sensitive periods. While ethical considerations prohibit assessment of alcohol sensitivity in human adolescents, animal studies can dissociate the consequences of heavy adolescent alcohol exposure from preceding or accompanying predispositions.

In animals, the discrete trials intracranial self-stimulation (ICSS) procedure is used to assess brain reward function. In this procedure, intracranial electrical stimulation is delivered contingent on an operant response. The rewarding electrical current is varied to determine the brain reward threshold, or minimal current intensity that maintains responding (Kornetsky et al., 1979; Markou & Koob 1992). This reward threshold is a direct measure of brain reward function. Acute administration of drugs that humans find rewarding lowers reward thresholds, reflecting enhanced brain reward function (Markou & Koob, 1992). Drug withdrawal, associated with a negative affective state in humans, elevates reward thresholds, reflecting brain reward deficits or anhedonia (Markou & Koob, 1991).

The literature on the effects of acute ethanol administration on brain reward function in adult rats without a history of previous ethanol exposure is equivocal, while ethanol withdrawal reliably produces threshold elevations (see Discussion). However, the long-term effects of adolescent ethanol exposure on brain reward function during re-exposure to ethanol in adulthood have not been studied. Interestingly, rodent strains bred for high ethanol consumption exhibit heightened sensitivity to the reward-enhancing effects of ethanol and blunted sensitivity to the anhedonia associated with ethanol withdrawal (Chester et al., 2006; Eiler et al., 2007; Fish et al., 2012). These findings suggest that heightened sensitivity to the reward-enhancing effects of ethanol or blunted sensitivity to the anhedonia of ethanol withdrawal accompany and may predict increased ethanol consumption. For example,

adolescent ethanol exposure, similar to selective breeding for high ethanol consumption, can result in increased adult ethanol self-administration under some conditions (Gilpin et al., 2012; Pascual et al., 2009; Sherrill et al., 2011). Based on the above findings, we hypothesized that adolescent ethanol exposure may decrease sensitivity to ethanol-induced anhedonia and may increase sensitivity to ethanol-induced reward enhancement in adulthood.

The goal of the present study was to investigate the long-term effects of adolescent intermittent ethanol (AIE) exposure on brain reward function in response to ethanol intoxication and ethanol withdrawal using the ICSS procedure. Human adolescence has been defined as the second decade of life and is characterized by hormonal, physiological, psychological and social changes marking the transition from childhood to adulthood. Rodent adolescence has been defined as occurring between post-natal day (PND) 28-42 (Spear, 2000). Others have considered the adolescent period to extend to PND 60 (Brenhouse & Andersen, 2011). Thus, we used a relatively broad definition of adolescence (PND 28-57) to ensure ethanol exposure during developmentally sensitive periods. We used an adolescent intermittent ethanol (AIE) exposure regimen consisting of experimenter-administered ethanol for 2 consecutive days, followed by 2 days of abstinence throughout adolescence to model the high levels of alcohol intoxication during alcohol binges reported in human adolescents. Similar intermittent adolescent ethanol exposure regimens in rodents have induced neurochemical and behavioral effects, such as neuroinflammation accompanied by reversal learning deficits (Vetreno & Crews, 2012), altered expression of dopamine receptors and extended dopamine release after ethanol (Pascual et al., 2009), and increased ethanol sensitivity of hippocampal extrasynaptic γ -aminobutyric acid-A (GABA_A) receptors (Fleming et al., 2012). Notably, adolescent binge-like intermittent ethanol exposure altered the affective valence of ethanol in adulthood as measured by increased ethanol self-administration (Alaux-Cantin et al., 2013; Pascual et al., 2009), and in conditioned taste aversion procedures (Alaux-Cantin et al., 2013). In the present work, the ICSS procedure was used to assess brain reward function in AIE-exposed and control rats during adulthood in response to acute ethanol, repeated daily ethanol withdrawals (to model human “hangover”), and withdrawal from a more severe 4-day ethanol binge.

EXPERIMENTAL PROCEDURES

Subjects

Timed-pregnant female Wistar rats (Charles River, Raleigh, NC) arrived in the vivarium on gestational day 13. Male pups were weaned on PND 21 and pair-housed in a humidity- and temperature-controlled vivarium on a 12 h/12 h reverse light/dark cycle. Food and water were available *ad libitum* at all times except when the rats were tested in the ICSS procedure. All of the procedures were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Adolescent intermittent ethanol exposure

From PND 28 to PND 57 (Cohort 1) or PND 28 to PND 53 (Cohort 2), the rats were administered 5 g/kg of 25% (v/v) ethanol or an equivalent volume of water intragastrically (IG) via oral gavage in a 2 days on/2 days off pattern. A relatively moderate AIE exposure regimen (a single ethanol administration on each binge day) was used in Cohort 1. In Cohort 2, a more severe AIE regimen (three ethanol administrations on each binge day at 8:00 AM, 12:00 PM, and 4:00 PM) was used. The rats were observed for behavioral intoxication signs (BISs) before each administration and doses were adjusted accordingly (Majchrowicz, 1975; Morris et al., 2010). As BISs increased, the subsequent ethanol dose was decreased (5-0 g/kg), such that a rat displaying no signs of intoxication received the full ethanol dose (5 g/kg). Rats that exhibited mild ataxia were given 4 g/kg. Rats that exhibited more severe ataxia were given 3 g/kg. Rats that displayed a delayed righting reflex received 2 g/kg. Rats that displayed a complete loss of the righting reflex received 1 g/kg and rats that displayed a loss of the eye blink reflex received no ethanol. Blood samples (200 µl) were taken from the tip of the tail for the analysis of blood ethanol concentrations (BECs) 60-90 min post-administration on binge days 8 and 16 (Cohort 1) and after the final administration on binge days 4, 8, and 14 (Cohort 2). Blood samples were immediately centrifuged at 1500 rpm for 15 minutes. Plasmas were then extracted and stored at -80°C until further analysis. Plasmas (5 µl) were analyzed using an Analox AM 1 analyzer (Analox Instruments LTD, Lunenburg, MA).

Surgical procedures

At least one week separated the final ethanol/water binge and surgical implantation of ICSS electrodes. Adult (PND 65-80) rats were anesthetized with an isoflurane/oxygen vapor mixture (1-3%). Stainless steel bipolar electrodes (11 mm, model MS303/2, Plastics One, Roanoke, VA) were implanted in the posterior lateral hypothalamus (anterior/posterior, -0.5 mm from bregma; medial/lateral, $\pm$ 1.7 mm; dorsal/ventral, -8.3 mm from dura; with the incisor bar 5 mm above the interaural line; Paxinos and Watson, 1998). At least one week was allowed for postoperative recovery before ICSS training was initiated.

Apparatus

ICSS testing was conducted in Plexiglas operant testing chambers (25 × 31 × 24 cm; Med Associates, St. Albans, VT) enclosed within sound- and light-attenuating boxes. A metal wheel (5 cm width, extending 3 cm) was located on one wall of the chamber. A quarter-turn of the wheel produced electrical stimulation through constant-current stimulators (Stimtek model 1200, Stimtek, Acton, MA) connected to the rat's electrode via flexible bipolar leads (Plastics One, Roanoke, VA) attached to gold-contact swivel commutators (model SL2C; Plastics One, Roanoke, VA) above the chamber. Experimental events were controlled by a computer.

Training and testing in the intracranial self-stimulation procedure

Rats were trained in the discrete-trials ICSS procedure modified from Kornetsky et al. (1979) as described in Markou and Koob (1992). Training consisted of initially reinforcing each quarter turn of the wheel before introducing the discrete-trials procedure. In the

discrete-trials procedure, stimulation was presented noncontingently, followed by a 7.5 s interval wherein the rat could make a wheel-turn response to receive identical stimulation. Each of these trials was separated by a variable-length timeout period with an average duration of 10 s. When the rats were reliably making wheel-turn responses, they were introduced to the threshold determination procedure. In each session, the stimulation current was varied in 5 μ A steps in alternating descending and ascending series (four series per session). Each stimulation current was presented in three trials. After three trials of identical current stimulation, the stimulation current was decreased (descending series) or increased (ascending series) by 5 μ A. A series threshold was defined as the mean of the first of two consecutive current intensities followed by a response on at least two of the three stimulus presentations and the first of two consecutive current intensities not followed by a response on at least two of the three trials. The mean of the four series thresholds was the threshold for that session. Testing in the discrete-trials current-threshold ICSS procedure continued for 6-7 days/week for at least 21 days until thresholds were stable (mean threshold variability across five consecutive sessions = $5.91\% \pm 0.86$ for Cohort 1 and $6.86\% \pm 0.47$ for Cohort 2 with no group differences in either cohort). Other recorded measures included mean response latency after a current stimulus presentation, number of extra responses within 2 s of a reinforced response (which had no scheduled consequences), and timeout responses (which occurred after this 2 s window and before the next stimulus presentation). Timeout responses resulted in a 10 s delay before the next trial.

Experimental design

Acute ethanol administrations—Once stable baseline ICSS thresholds were established, the response to acute ethanol (25% v/v via oral gavage) was assessed. Rats in Cohort 1 were between PND 135-167 when they were administered four ethanol doses (0.3, 0.75, 1.5, and 3 g/kg). Because the liquid volume varied by a factor of 10 from the smallest to the largest ethanol dose, two vehicle volumes were also administered, equal to the volume of ethanol administered at the highest (3 g/kg) and lowest (0.3 g/kg) ethanol doses. ICSS testing was conducted at five time-points within 24 h of administration (5 min, 3 h, 6 h, 12 h, and 24 h post- **administration**). The ethanol doses used for Cohort 2 (0.75, 1.5, and 2 g/kg and a single vehicle volume) were adjusted based on Cohort 1 results. Rats in Cohort 2 were between PND 120-146 for the acute ethanol administrations. Cohort 2 rats were tested at three time-points (5 min, 6 h, and 24 h post- administration). For both cohorts, at least three days of stable baseline thresholds separated each administration. After completing the ICSS session (approximately 30-40 min post- administration), tail-tip blood samples were collected for BEC analysis.

Daily ethanol administrations—After all acute ethanol challenges, stable thresholds were reestablished, and rats were daily administered 4 g/kg ethanol (25% v/v) or an equivalent volume of water via oral gavage and tested 24 h later for 5 (Cohort 1, PND 168-172) or 6 consecutive days (Cohort 2, PND 150-156).

Four-day binge—The rats were administered a 4-day binge of either three equally spaced administrations each day (8:00 AM, 4:00 PM, and 12:00 AM; Cohort 1, PND 204-216) or two administrations each day (8:00 AM and 6:00 PM; Cohort 2, PND 167-177). Rats that

received water in the previous experimental phase were administered ethanol (25% v/v), whereas rats that previously received ethanol were administered water. For both cohorts, a 5 g/kg priming dose was first administered, with subsequent doses adjusted according to BISs (see above). Rats were tested in the ICSS task 12 h after the final dose and at 24 h intervals thereafter.

Statistical analyses

Reward thresholds are expressed as percent change from the preceding stable 3-5 day baseline threshold, with values less than 10% indicating reward enhancement and values greater than 10% indicating reward deficits. No threshold differences were observed after administration of the small and large vehicle volumes in Cohort 1. Therefore, data from these two vehicle administration sessions were combined. All group data were subjected to univariate analysis of variance (ANOVA) using SPSS 18 (SPSS, Chicago, IL, USA). For the acute administrations, two-way ANOVAs were used, with *Group* (AIE vs. control) as the between-subjects factor and *Ethanol Dose* as the within-subjects factor. For the daily administrations, separate two-way ANOVAs were performed on AIE-exposed and control rats, with *Daily Treatment* (ethanol vs. water) as the between-subjects factor and *Day* as the within-subjects factor. For the 4-day binge, a three-way ANOVA was performed, with *Group* (AIE vs. control) and *Binge Treatment* (ethanol vs. water) as the between-subjects factors and *Timepoint* as the within-subjects factor. ANOVAs were also conducted with these factors for the other performance measures (extra responses, timeout responses, and response latency). The level of significance was set at 0.05. Significant main effects and interactions were followed by simple-effects ANOVAs and *t*-tests using a Šidák adjustment for multiple comparisons. For repeated-measures analyses, Mauchly's test of sphericity of the covariance matrix was applied. When the sphericity assumption was violated, the degrees of freedom for any term involving that factor were adjusted to more conservative values by applying the Huynh-Feldt correction, reported to two decimal places. To evaluate individual differences in response to acute ethanol, non-parametric χ^2 analyses were conducted comparing the percentage of control and AIE-exposed rats exhibiting thresholds at least 10% lower than baseline after any ethanol dose.

RESULTS

Ethanol administered and blood ethanol concentrations during adolescent intermittent ethanol exposure

Cohort 1 AIE-exposed rats were always administered 5 g/kg ethanol each binge day. Binge BECs significantly increased from binge day 8 to 16 (92.57 ± 8.87 mg/dl and 172.13 ± 9.97 mg/dl, respectively; *t*-test: $t_8 = 7.49$, $p < 0.001$). In Cohort 2, the ethanol dose received by the rats during binges ranged from 26.83 to 28.88 (average 27.59). There was a significant effect of *PND* on ethanol dose administered ($F_{5.09, 198.37} = 10.27$, $p < .001$). *Post hoc* *t*-tests revealed only that more ethanol was administered during the second binge ($28.88 \pm .176$ g/kg) relative to all other binges (range = $26.83 \pm .27$ to $27.90 \pm .28$). There were no other significant differences between binges (Supplementary data Figure S1). Binge BECs were 252.76 ± 17.32 mg/dl, 393.20 ± 19.45 mg/dl, and 436.95 ± 15.00 mg/dl on binge days 4, 8, and 14, respectively. There was a significant main effect of *Binge Day* ($F_{2,26} = 31.63$, $p <$

0.001) on binge BECs. *Post hoc t*-tests indicated a lower BEC on binge day 4 than on binge days 8 and 14 ($p < 0.001$). Cohort 1 body weights were not affected by the moderate AIE exposure, whereas Cohort 2 rats exposed to the severe AIE regimen had significantly lower body weights throughout adolescence, with a nonsignificant trend in this direction during adulthood (Supplementary Table S1).

Baseline ICSS performance

In Cohort 1, baseline thresholds did not differ between control ($132.34 \pm 17.43 \mu\text{A}$) and AIE-exposed ($132.85 \pm 15.33 \mu\text{A}$) rats. Additionally, no group differences were found in mean response latencies (Control: 3.36 ± 0.37 s; AIE: 3.50 ± 0.33 s), extra responses (Control: 15.29 ± 11.90 ; AIE: 19.49 ± 30.45), or timeout responses (Control: 9.11 ± 5.08 ; AIE: 10.91 ± 5.48). Similarly, in Cohort 2, no differences were found in baseline thresholds (Control: $149.43 \pm 13.66 \mu\text{A}$; AIE: $147.58 \pm 13.19 \mu\text{A}$), mean response latencies (Control: 3.42 ± 0.07 s; AIE: 3.34 ± 0.13 s), extra responses (Control: 29.22 ± 5.82 ; AIE: 22.14 ± 6.26), or timeout responses (Control: 15.14 ± 2.22 ; AIE: 9.66 ± 2.27).

Acute ethanol administrations

There was no main effect on ICSS thresholds or interaction involving the factor *Group* (control vs. AIE) in Cohort 1 rats after acute ethanol. There was a significant main effect of *Dose* ($F_{2,82,50.81} = 13.91$, $p < .001$). *Post hoc* tests indicated elevated reward thresholds in control and AIE-exposed rats after the highest ethanol dose (3 g/kg; Fig. 1A). A nonsignificant trend was observed for less pronounced threshold elevations in AIE-exposed rats compared to control rats at all ethanol doses. In addition, there was high inter-subject variability in thresholds in response to lower ethanol doses (0.3-1.5 g/kg). Analysis of individual differences were performed to determine the percent of rats that showed threshold lowering (reward thresholds at least 10% less than baseline) at the lower ethanol doses (0.3-1.5 g/kg), although the threshold-lowering dose varied across individual rats. The proportion of rats that exhibited threshold lowering after at least one ethanol dose (Fig. 1B) was significantly higher in AIE-exposed (56%; five of nine) compared with control rats (9%; one of 11; $\chi^2_{1,20} = 5.09$, $p < 0.05$). When tested at later time-points (Supplementary Fig. S2), AIE-exposed and control rats showed similar threshold elevations after the highest ethanol doses (1.5 and 3 g/kg). Lower doses (0.3 and 0.75 g/kg) had no effect on thresholds at any time-point in either group (Supplementary Fig. S2).

For Cohort 2 (Fig. 1C), a significant *Group* $\times$ *Dose* interaction was observed ($F_{3,108} = 3.48$, $p < 0.05$). *Post hoc* tests indicated greater threshold elevations in control rats after 2 g/kg ethanol. One of the 15 AIE-exposed and none of the control rats showed threshold lowering exceeding 10% after any ethanol dose (Fig. 1D). No group or dose effects were found at later time-points (Supplementary Fig. S3).

There was no effect of *Group* on any of the three other response measures (extra responses, timeout responses, response latencies) in either cohort. However, independent of AIE exposure, there was a significant main effect of *Ethanol Dose* on extra responses in Cohort 1 ($F_{2,00,34.04} = 3.39$, $p < 0.05$) and Cohort 2 ($F_{3,108} = 4.65$, $p < 0.005$). In Cohort 1, *post hoc* tests indicated more extra responses after 3 g/kg ethanol (26.86 ± 9.54) than after vehicle

(16.42 ± 5.38). In Cohort 2, *post hoc* tests indicated more extra responses after 1.5 g/kg ethanol (46.00 ± 8.81) and 2 g/kg ethanol (41.18 ± 6.65) than after vehicle (29.92 ± 5.23). No group differences in BECs were observed in either cohort (Supplementary Fig. S4).

Daily ethanol administrations (“hangover” effect)

There was a significant main effect of *Treatment* on thresholds in control rats from Cohort 1 (Fig. 2A, $F_{1,9} = 8.37$, $p < 0.05$) and Cohort 2 (Fig. 2C, $F_{1,14} = 11.86$, $p < 0.05$). In both cohorts control rats administered ethanol showed higher thresholds than control rats administered water. A significant *Day* $\times$ *Treatment* interaction was also observed in Cohort 2 control rats ($F_{5,70} = 2.55$, $p < 0.05$). *Post hoc* tests indicated significantly higher thresholds in control rats administered ethanol compared with control rats administered water on days 2 and 3, with further nonsignificant trends ($p < 0.08$) in this direction on days 4 and 6. There was no effect of *Treatment* in AIE-exposed rats in Cohort 1 (Fig. 2B) or Cohort 2 (Fig. 2D). No meaningful significant differences among the three other response measures (extra responses, timeout responses, response latencies) were found in either cohort.

Four-day binge exposure

Supplementary Table S2 presents total ethanol administered, average BIS, and BEC 60 min after the final ethanol administration in Cohort 1 and Cohort 2 rats exposed to a 4-day ethanol binge. A significant main effect of *Cohort* was found for all three measures. BISs and BECs were higher in Cohort 1 (BIS: $F_{1,24} = 358.75$, $p < 0.001$; BEC: $F_{1,24} = 19.10$, $p < 0.001$), while Cohort 2 rats received more ethanol ($F_{1,24} = 17.49$, $p < 0.001$). No effect of *AIE* and no *Cohort* $\times$ *AIE* interactions were found for any measure.

During withdrawal there was a significant *Timepoint* $\times$ *Binge* interaction ($F_{7,66,99.56} = 14.10$, $p < 0.001$), with no effect of *AIE* in Cohort 1. *Post hoc* tests confirmed that thresholds were elevated for 6 days in rats exposed to a 4-day ethanol binge compared with rats exposed to a water binge, independent of *AIE* exposure (Fig. 3). A significant *Timepoint* $\times$ *Binge* interaction was observed in Cohort 2 ($F_{3,22,8.17} = 8.17$, $p < 0.001$). *Post hoc* tests revealed that rats exposed to a 4-day ethanol binge showed elevated thresholds 12–24 h after the binge. Though there was no effect of *AIE*, exploratory comparisons of control and AIE-exposed rats revealed larger threshold elevations in control than in AIE-exposed rats 24 h after the ethanol binge. No meaningful significant differences among the three other measures (extra responses, timeout responses, response latencies) were observed in either cohort.

DISCUSSION

Adolescent intermittent ethanol (AIE) exposure altered adult brain reward function in response to acute ethanol and ethanol withdrawal. In control rats, acute ethanol elevated reward thresholds, suggesting anhedonia. Binge-like ethanol exposure during adolescence diminished reward deficits induced by acute alcohol administration. Furthermore, analysis of individual differences in response to ethanol revealed that a sub-set of AIE-exposed rats showed ethanol-induced reward enhancement, rather than ethanol-induced reward deficits. AIE exposure also diminished threshold elevations 24 h after 4 g/kg ethanol, indicating

decreased negative affect during ethanol withdrawal (“hangover”). Lastly, although control and AIE-exposed rats exhibited similar threshold elevations after a severe 4-day ethanol binge, AIE-exposed rats showed diminished threshold elevations after a moderate 4-day ethanol binge compared to rats treated with vehicle in adolescence, again suggesting reduced reward deficits in AIE-exposed rats. Altogether, these findings suggest that adolescent ethanol exposure may decrease sensitivity to the anhedonic, aversive effects of ethanol withdrawal. Additionally, while the group mean data revealed no significant differences in the reward-enhancing effects of ethanol, exploratory analyses suggested that adolescent intermittent ethanol exposure may perhaps also increase sensitivity to the rewarding or appetitive effects of ethanol. Rodents selectively bred for high ethanol consumption show similar ethanol sensitivities (Chester et al., 2006; Eiler et al., 2007; Fish et al., 2012), suggesting that high sensitivity to ethanol reward and low sensitivity to ethanol withdrawal may predict heavy or problematic alcohol use.

The threshold elevations after acute ethanol seen in control rats indicate that anhedonia accompanies ethanol intoxication. The literature on brain reward function during ethanol intoxication is equivocal. While a number of studies, consistent with the present findings, report decreased (e.g., Schaefer & Michael, 1992) or unaltered brain reward function (e.g., Moolten & Kornetsky, 1990; Schaefer & Michael, 1987) by ethanol, other studies report ethanol-induced reward enhancement (e.g., Bain & Kornetsky, 1989; Beshpalov et al., 1999; de Witte & Bada, 1983; Eiler et al., 2007; Fish et al., 2012; Lewis & June, 1990, 1994). Some of these discrepancies may be attributable to different experimental procedures. Enhanced brain reward function by acute ethanol in mice (e.g., Fish et al., 2012) and selectively bred alcohol-preferring rats (Eiler et al., 2007) may not generalize to outbred rat strains. Additionally, some studies used uncommon ICSS procedures (Lewis & June, 1990, 1994; Musgrave et al., 1989) or inferred reward enhancement from increased response rates (rate-frequency procedures; Bain & Kornetsky, 1989; de Witte & Bada, 1983). In the present study, we assessed the effects of ethanol on brain reward function using the rate-free discrete-trials current-threshold procedure (Kornetsky et al., 1979; Markou & Koob, 1992). This procedure provides independent measures of reward (reward threshold) and motor processes (response latency and extra responses). The rate-frequency and uncommon ICSS procedures used in other studies leave open the possibility that the observed effects were attributable to motor stimulation and not reward enhancement. Consistent with this possibility, in the present study, ethanol increased extra responses (responses within 2 s of a reinforced response) at doses that elevated reward thresholds, suggesting motor-stimulating effects that are distinct and dissociable from any effects on reward processing. Thus, earlier studies using procedures that did not account for these motor-stimulatory effects of ethanol may confound reward enhancement with motor stimulation.

Reward enhancement by acute ethanol in adult outbred rats has been demonstrated in ICSS procedures similar (Beshpalov et al., 1999) or identical to that used in the present study (Moolten & Kornetsky, 1990). Notably, Moolten and Kornetsky (1990) observed enhanced brain reward function by orally self-administered ethanol at doses which produced threshold elevations when administered via implanted gastric cannulae. These findings suggest that the hedonic value of specific ethanol doses may vary when the ethanol is experimenter-administered in a single bolus dose from when the ethanol is self-administered, and thus

self-regulated, across a self-administration session, even when the total cumulative ethanol dose received by the subject is identical. Nevertheless, threshold elevations after experimenter-administered ethanol are consistent with the threshold elevations observed in control rats in the present study and demonstrate that experimenter-administered ethanol induces anhedonia in nondependent adult outbred rats.

AIE exposure reduced the anhedonia associated with acute ethanol intoxication. In Cohort 1, the ethanol dose-response curve did not reveal different reward thresholds in control and moderately AIE-exposed rats. However, analysis of individual differences analyses revealed that moderate AIE exposure increased the likelihood that rats would exhibit threshold lowering, rather than elevation, after acute ethanol. Ethanol-induced reward enhancement was not apparent in the dose-response function because the effective ethanol dose varied across individual rats (0.3-1.5 g/kg), a finding consistent with the variability in threshold-lowering ethanol dose (0.8-1.6 g/kg) among rats permitted to orally self-administer ethanol before ICSS testing (Moolten & Kornetsky, 1990). The highest ethanol dose used in Cohort 1 (3 g/kg) induced threshold elevations in AIE-exposed and control rats, indicating that rats administered a moderate AIE regimen showed some reward deficits after high -dose ethanol. In contrast, Cohort 2 rats showed neither reward enhancement nor reward deficits by acute ethanol suggesting that severe AIE exposure blunts the response to the reward-enhancing and anhedonic effects of ethanol.

Consistent with previous findings (Chester et al., 2006; de Witte & Bada, 1983; Schulteis & Liu, 2006), daily ethanol (4 g/kg) produced threshold elevations 24 h after administration in control rats in both cohorts, reflecting the anhedonia of ethanol withdrawal. In contrast, independent of exposure severity, AIE-exposed rats showed no threshold elevations, indicating diminished reward deficits during withdrawal from modest ethanol exposure ("hangover"). Rats selectively bred for high alcohol drinking also showed diminished threshold elevations during withdrawal from similar ethanol doses (Chester et al., 2006).

When tested during withdrawal from a severe 4-day ethanol binge which engendered high BECs and visible behavioral intoxication, Cohort 1 AIE-exposed and control rats showed similar threshold elevations. Thresholds peaked at 60% greater than baseline and remained elevated for 6 days in all rats. The less severe 4-day ethanol binge used in Cohort 2 resulted in lower BECs and BISs compared to Cohort 1. In Cohort 2 control and AIE-exposed rats, thresholds were elevated only 30% above baseline and returned to baseline within 2 days. Interestingly, Cohort 2 AIE-exposed rats showed decreased magnitude of ethanol withdrawal 24 h after the ethanol binge compared to control rats. The diminished anhedonia observed in Cohort 2 AIE-exposed rats cannot be attributed to physiological tolerance to ethanol because there were no differences between AIE-exposed and control rats in behavioral intoxication or blood ethanol concentration during the 4-day ethanol binge.

Disturbed functioning of both the dopamine and corticotrophin releasing factor (CRF) systems may account for the altered sensitivity to ethanol and ethanol withdrawal in AIE-exposed rats. Extensive interactions exist between brain dopamine and CRF (George et al., 2012). For example, dopamine projections innervate brain structures rich in CRF expression (Meloni et al., 2006) while CRF increases dopamine release (Lavicky and Dunn, 1993).

Ethanol administration has effects on both of these brain systems: dopamine transmission is central to the reinforcing effects of ethanol (Imperato & Di Chiara, 1986) and acutely administered ethanol stimulates CRF release (Rivier et al., 1984). Notably, rats exposed to ethanol during adolescence show elevated dopamine levels in the nucleus accumbens at baseline (Badanich et al., 2007; Pascual et al., 2009; Philpot et al., 2009) and after acute ethanol (Pascual et al., 2009). Elevated basal dopamine and a heightened dopamine response to ethanol may account for the threshold lowering after acute ethanol in some AIE-exposed Cohort 1 rats. In contrast, decreased dopamine is associated with ethanol withdrawal (Rossetti et al., 1992) and alcohol craving severity in detoxified alcoholic patients (Heinz et al., 2005). The chronically elevated basal dopamine after adolescent ethanol exposure (Badanich et al., 2007) may have alleviated the ethanol withdrawal-induced brain reward deficits associated with decreased dopamine. CRF is critically involved in the anhedonia associated with acute ethanol intoxication and ethanol withdrawal (Valdez et al., 2002). Specifically, CRF administration induces ICSS threshold elevations (Macey et al., 2000) and threshold elevations during ethanol withdrawal are reduced by a CRF antagonist (Bruijnzeel et al., 2010). Notably, adolescent ethanol exposure attenuates the adult CRF response to ethanol (Allen et al., 2011). A blunted CRF ethanol response may partly explain the diminished threshold elevations in response to acute ethanol and ethanol withdrawal (“hangover”) in AIE-exposed rats in the present study.

In summary, the present study found that binge-like ethanol exposure during adolescence diminishes the aversive anhedonic effects of ethanol and may enhance the appetitive reward-enhancing effects of ethanol in a sub-set of subjects in adulthood in male Wistar rats. In humans, high response to the appetitive effects and low response to the aversive effects of ethanol predict the later development of AUDs (King et al., 2011). Thus, our findings suggest that altered affective response to acute ethanol intoxication and ethanol withdrawal may increase ethanol consumption, perhaps increasing the risk of developing an AUD in humans. Further research will determine whether these results are unique to ethanol exposure during the developmentally sensitive adolescent period and whether these conclusions extend to females.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

- Allen CD, Rivier CL, Lee SY. Adolescent alcohol exposure alters the central brain circuits known to regulate the stress response. *Neuroscience*. 2011; 182:162–168. [PubMed: 21382450]
- Alaux-Cantin S, Warnault V, Legastelois R, Botia B, Pierrefiche O, Vilpoux C, Naasila M. Alcohol intoxications during adolescence increase motivation for alcohol in adult rats and induce neuroadaptations in the nucleus accumbens. *Neuropharmacology*. 2013; 67:521–531. [PubMed: 23287538]
- Badanich KA, Maldonado AM, Kirstein CL. Chronic ethanol exposure during adolescence increases basal dopamine in the nucleus accumbens septi during adulthood. *Alcohol Clin Exp Res*. 2007; 31:895–900. [PubMed: 17391340]
- Bain GT, Kornetsky C. Ethanol oral self-administration and rewarding brain stimulation. *Alcohol*. 1989; 6:499–503. [PubMed: 2597352]

- Bespalov A, Lebedev A, Panchenko G, Zvartau E. Effects of abused drugs on thresholds and breaking points of intracranial self-stimulation in rats. *Eur Neuropsychopharmacol.* 1999; 9:377–383. [PubMed: 10523044]
- Brenhouse HC, Andersen SL. Developmental trajectories during adolescence in males and females: A cross-species understanding of underlying brain changes. *Neurosci Biobehav Rev.* 2011; 35:1687–1703. [PubMed: 21600919]
- Bruijnzeel AW, Small E, Pasek TM, Yamada H. Corticotropin-releasing factor mediates the dysphoria-like state associated with alcohol withdrawal in rats. *Behav Brain Res.* 2010; 210:288–291. [PubMed: 20193713]
- Chester JA, Rausch EJ, June HL, Froehlich JC. Decreased reward during acute alcohol withdrawal in rats selectively bred for low alcohol drinking. *Alcohol.* 2006; 38:165–172. [PubMed: 16905442]
- de Witte P, Bada MF. Self-stimulation and alcohol administered orally or intraperitoneally. *Exp Neurol.* 1983; 82:675–682. [PubMed: 6653716]
- Eiler WJA 2nd, Hardy L 3rd, Goergen J, Seyoum R, Mensah-Zoe B, June HL. Responding for brain stimulation reward in the bed nucleus of the stria terminalis in alcohol-preferring rats following alcohol and amphetamine pretreatments. *Synapse.* 2007; 61:912–924. [PubMed: 17701965]
- Fish EW, Robinson JE, Krouse MC, Hodge CW, Reed C, Phillips TJ, Malanga CJ. Intracranial self-stimulation in FAST and SLOW mice: effects of alcohol and cocaine. *Psychopharmacology.* 2012; 220:719–730. [PubMed: 21983918]
- Fleming RL, Acheson SK, Moore SD, Wilson WA, Swartzwelder HS. In the rat, chronic intermittent ethanol exposure during adolescence alters the ethanol sensitivity of tonic inhibition in adulthood. *Alcohol Clin Exp Res.* 2012; 36:279–285. [PubMed: 22014205]
- George O, Le Moal M, Koob GF. Allostasis and addiction: Role of the dopamine and corticotropin-releasing factor systems. *Physiology & Behavior.* 2012; 106:58–64. [PubMed: 22108506]
- Gilpin NW, Karanikas CA, Richardson HN. Adolescent binge drinking leads to changes in alcohol drinking, anxiety, and amygdalar corticotropin releasing factor cells in adulthood in male rats. *PLoS One.* 2012; 7:e31466. [PubMed: 22347484]
- Grant BF, Dawson DA. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse.* 1997; 9:103–110. [PubMed: 9494942]
- Heinz A, Siessmeier T, Wrase J, Buchholz HG, Gründer G, Kumakura Y, Cumming P, Schreckenberger M, Smolka MN, Rösch F, Mann K, Bartenstein P. Correlation of alcohol craving with striatal dopamine synthesis capacity and D_{2/3} receptor availability: a combined [¹⁸F]DOPA and [¹⁸F]DMFP PET study in detoxified alcoholic patients. *Am J Psychiatry.* 2005; 162:1515–1520. [PubMed: 16055774]
- Hibell B, Guttormsson U, Ahlström S, Balakireva O, Bjarnason T, Kokkevi A, Kraus L. The 2007 ESPAD Report: Substance Use Among Students in 35 European Countries. The Swedish Council for Information on Alcohol and Other Drugs; Stockholm: 2009.
- Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther.* 1986; 239:219–228. [PubMed: 3761194]
- Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the Future: National Results on Adolescent Drug Use: Overview of Key Findings, 2012. Ann Arbor; Institute for Social Research, University of Michigan: 2013.
- King AC, de Wit H, McNamara PJ, Cao D. Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. *Arch Gen Psychiatry.* 2011; 68:389–399. [PubMed: 21464363]
- Kornetsky C, Esposito RU, McLean S, Jacobson JO. Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. *Arch Gen Psychiatry.* 1979; 36:289–292. [PubMed: 420547]
- Lavicky J, Dunn AJ. Corticotropin-Releasing Factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. *J Neurochem.* 1993; 60:602–612. [PubMed: 7678287]
- Lewis MJ, June HL. Neurobehavioral studies of ethanol reward and activation. *Alcohol.* 1990; 7:213–219. [PubMed: 2184834]

- Lewis MJ, June HL. Synergistic effects of ethanol and cocaine on brain stimulation reward. *J Exp Anal Behav.* 1994; 61:223–229. [PubMed: 8169571]
- Macey DJ, Koob GF, Markou A. CRF and urocortin decreased brain stimulation reward in the rat: reversal by a CRF receptor antagonist. *Brain Res.* 2000; 866:82–91. [PubMed: 10825483]
- Majchrowicz E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia.* 1975; 43:245–254. [PubMed: 1237914]
- Markou A, Koob GF. Postcocaine anhedonia: an animal-model of cocaine withdrawal. *Neuropsychopharmacology.* 1991; 4:17–26. [PubMed: 2003866]
- Markou A, Koob GF. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol Behav.* 1992; 51:111–119. [PubMed: 1741436]
- Meloni EG, Gerety LP, Knoll AT, Cohen BM, Carlezon WA. Behavioral and anatomical interactions between dopamine and Corticotropin-Releasing Factor in the rat. *J Neurosci.* 2006; 26:3855–3863. [PubMed: 16597740]
- Moolten M, Kornetsky C. Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. *Alcohol.* 1990; 7:221–225. [PubMed: 2184835]
- Morris SA, Kelso ML, Liput DJ, Marshall SA, Nixon K. Similar withdrawal severity in adolescents and adults in a rat model of alcohol dependence. *Alcohol.* 2010; 44:89–98. [PubMed: 20113877]
- Pascual M, Boix J, Felipe V, Guerri C. Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. *J Neurochem.* 2009; 108:920–931. [PubMed: 19077056]
- Philpot RM, Wecker L, Kirstein CL. Repeated ethanol exposure during adolescence alters the developmental trajectory of dopaminergic output from the nucleus accumbens septi. *Int J Dev Neurosci.* 2009; 27:805–815. [PubMed: 19712739]
- Rivier C, Bruhn T, Vale W. Effect of ethanol on the hypothalamic-pituitary-adrenal axis in the rat: role of corticotropin-releasing factor (CRF). *J Pharmacol Exp Ther.* 1984; 229:127–131. [PubMed: 6323684]
- Rossetti ZL, Melis F, Carboni S, Diana M, Gessa GL. Alcohol withdrawal in rats is associated with a marked fall in extraneuronal dopamine. *Alcohol Clin Exp Res.* 1992; 16:529–532. [PubMed: 1626652]
- Schaefer GJ, Michael RP. Ethanol and current thresholds for brain self-stimulation in the lateral hypothalamus of the rat. *Alcohol.* 1987; 4:209–213. [PubMed: 3593536]
- Schaefer GJ, Michael RP. Interactions between alcohol and nicotine on intracranial self-stimulation and locomotor activity in rats. *Drug Alcohol Depend.* 1992; 30:37–47. [PubMed: 1591979]
- Schuckit MA. Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry.* 1994; 151:184–189. [PubMed: 8296886]
- Schulteis G, Liu J. Brain reward deficits accompany withdrawal (hangover) from acute ethanol in rats. *Alcohol.* 2006; 39:21–28. [PubMed: 16938626]
- Schulteis G, Markou A, Cole M, Koob GF. Decreased brain reward produced by ethanol withdrawal. *Proc Natl Acad Sci U S A.* 1995; 92:5880–5884. [PubMed: 7597046]
- Sherrill LK, Koss WA, Foreman ES, Gulley JM. The effects of pre-pubertal gonadectomy and binge-like ethanol exposure during adolescence on ethanol drinking in adult male and female mice. *Behav Brain Res.* 2011; 216:569–575. [PubMed: 20816899]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev.* 2000; 24:417–463. [PubMed: 10817843]
- Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, Koob GF. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. *Alcohol Clin Exp Res.* 2002; 26:1494–1501. [PubMed: 12394282]
- Vetreno RP, Crews FT. Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and Toll-like receptors in the adult prefrontal cortex. *Neuroscience.* 2012; 226:475–488. [PubMed: 22986167]

Figure 1.

ICSS thresholds after acute ethanol in Cohort 1 (moderate AIE exposure) (A) and Cohort 2 (severe AIE exposure) (C) and percentage of Cohort 1 (B) and Cohort 2 (D) rats in each group that showed threshold lowering at least 10% relative to baseline after any acute ethanol dose. The data are expressed as mean $\pm$ SEM. The carat symbol indicates a significant difference ($p < 0.05$; *post hoc t*-test with Šidák correction for multiple comparisons) compared with vehicle in both AIE-exposed and control rats. The hash symbol denotes a significant difference ($p < 0.05$; *post hoc t*-test with Šidák correction for multiple comparisons) between control and AIE-exposed rats. The asterisk denotes a significant difference (χ^2 test) in the number of rats in each group that exhibited threshold lowering that exceeded 10%. Notice the different y-axis scales in panel A (Cohort 1) and panel C (Cohort 2).

Figure 2.

ICSS thresholds after 4 g/kg daily ethanol or water administration in control rats in Cohort 1 (A) and Cohort 2 (C) and AIE-exposed rats in Cohort 1 (B) and Cohort 2 (D). The data are expressed as group mean $\pm$ SEM. (A) The asterisk denotes a significant difference between ethanol- and water-treated control rats (significant main effect of daily ethanol vs. daily water; $p < 0.05$). (C) The asterisks denote significant differences between ethanol- and water-treated control rats ($p < 0.05$; *post hoc t*-test with Sidak correction for multiple comparisons). No differences in thresholds were found between ethanol-treated and water-treated AIE-exposed rats in Cohort 1 or 2.

Figure 3.

ICSS thresholds after a 4-day ethanol or water binge in control and AIE-exposed rats in Cohort 1 (A) and Cohort 2 (B). The data are expressed as group mean $\pm$ SEM. The caret symbols indicate a significant difference between ethanol binge-exposed and water binge-exposed rats, independent of AIE exposure (*post hoc t*-tests with Šidák adjustment for multiple comparisons, $p < 0.05$). The asterisk denotes a significant difference between AIE-exposed and control rats treated with an ethanol binge (*t*-test with Šidák adjustment for multiple comparisons, $p < 0.05$).