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Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats

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Abstract

Elevated signs of anxiety are observed in adult rodents during withdrawal from chronic as well as acute ethanol exposure. To determine whether adolescents, in addition to their insensitivity to a number of acute ethanol effects, might likewise be hyposensitive to these anxiogenic manifestations of withdrawal from an acute ethanol challenge, the behavior of adolescent and adult male Sprague–Dawley rats was assessed in an elevated plus maze (EPM) 18 h following intraperitoneal challenge with 4 g/kg ethanol. Adult but not adolescent animals demonstrated evidence of anxiety in the plus maze during acute ethanol withdrawal. To ensure that this finding did not merely reflect age differences in ethanol clearance, clearance times at each age were determined, with additional adolescents tested at the same time postclearance as the adults were previously. Adolescents still failed to demonstrate anxiogenic signs of withdrawal. Suppression of activity during the withdrawal test, however, was evident in animals of both ages. A relative resistance to the anxiogenic effects associated with acute ethanol withdrawal during adolescence could serve as a permissive factor for development of binge drinking patterns among human adolescents.

Keywords: Acute withdrawal; Adolescence; Anxiety; Ethanol; Rat

1. Introduction

Alcohol use remains a prevalent problem among American adolescents. According to the Monitoring the Future Study of 2000, 52% of 8th graders and 80% of 12th graders reported having tried alcohol at least once in their life (Johnston et al., 2001). Of these same teens, 22% of 8th graders and 50% of 12th graders reported having had alcohol within the past month. Even more alarming is the percentage of teens that reported drinking in large amounts. Heavy episodic drinking, termed ‘‘binge drinking’’ by the Monitoring the Future Study, has been defined as five or more drinks in a row. Fourteen percent of 8th graders, 26% of 10th graders, and 30% of 12th graders said that by this criterion, they engaged in ‘‘binge drinking’’ during the past 2 weeks.

The reasons why adolescent alcohol use remains so prevalent still are not clear. One possibility is that adolescents may exhibit insensitivity to some of the negative consequences of alcohol use, both during and after consumption. Current research using animal models provides some support for this hypothesis, with adolescent animals being less sensitive than adults to a number of ethanol effects, including its hypnotic (Little et al., 1996; Silveri and Spear, 1998), hypothermic (Silveri and Spear, 2000), motor impairing (White et al., 2002) and anxiolytic (Varlinskaya and Spear, 2002a) effects. For instance, Silveri and Spear (1998) demonstrated that adolescent rats regained their righting reflex more quickly and woke at higher blood alcohol levels (BALs) than more mature animals following a large acute dose of ethanol. As another example, White et al. (2002) found adolescent rats to be less impaired on a tilting plane test than adult rats at higher doses (2.0 or 3.0 g/kg), but not at a low dose (1.0 g/kg) of ethanol. Conversely, adolescents are more sensitive to certain restricted effects of ethanol, including ethanol-induced impairments in hippocampal LTP and NMDA receptor function (Swartzwelder et al., 1995a,b), as well as ethanol-related disruption in performance on a spatial memory task (Markwiese et al., 1998). These instances of greater sensitivities seemingly would be less readily perceived by adolescents and hence less likely to serve as cues to moderate intake than consequences of

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ethanol consumption to which adolescents are relatively resistant, such as sedative and motor impairing effects.

Researchers have hypothesized that symptoms of hangover and withdrawal may help to prevent further abuse of the drug on subsequent occasions (e.g., Smith and Barnes, 1983). Although an insensitivity to the negative consequences following episodes of drinking could support patterns of further binge drinking, such as those seen among adolescents, little research has been done to examine whether adolescence is a period of greater or lessened sensitivity to these effects of prior ethanol use.

The symptomatic profile of withdrawal has been well established in both human and animal subjects. Signs of withdrawal from ethanol are most severe early in the withdrawal period following extended periods of alcohol consumption, with signs that include nausea, tremors, hyperthermia, tachycardia, irritability, hyperventilation, anxiety and insomnia (Roelofs, 1985; for reviews, see Finn and Crabbe, 1997; Metten and Crabbe, 1996). A milder form of withdrawal, called acute withdrawal (or hangover) can occur following a single episode of drinking. Physiological symptoms of hangover in humans include headache, nausea, diarrhea, anorexia, fatigue, and tremor (Bogin et al., 1986; for reviews, see Weise et al., 2000; Smith and Barnes, 1983), along with psychological signs that include anxiety, guilt, and depression (Bogin et al., 1986; Smith and Barnes, 1983). Although lower than the incidence of anxiety during withdrawal after prolonged ethanol use, reports of anxiety during hangover are also significant, with as many as 20% of adult humans reporting signs of anxiety during hangovers (Smith and Barnes, 1983).

Models of ethanol withdrawal in adult rodents have consistently demonstrated increases in anxiety during the withdrawal period after chronic exposure to ethanol (File, 1994; File et al., 1991, 1993; Gatch et al., 1999; Lal et al., 1991; for a review, see Gatch and Lal, 2001). For instance, adult rats chronically exposed to ethanol and tested during ethanol withdrawal spent less percentage of time on the open arms of the elevated plus maze (EPM) and made a smaller percentage of entries into the open arms (Lal et al., 1991), a response pattern consistent with an anxiogenic profile (Pellow et al., 1985). Increases in anxiety during withdrawal from ethanol have also been observed in other behavioral tests, including a light–dark box, open field, holeboard test, social interaction test, and pentylenetetrazol (PTZ) drug discrimination (for reviews, see Becker, 2000; Gatch and Lal, 2001). A conditioned place aversion paradigm has demonstrated aversive signs of withdrawal in rats following an acute exposure to ethanol (Morse et al., 2000), but to our knowledge there are no reports that have examined the anxiogenic response of withdrawal following a single dose of ethanol on the EPM in either adult or adolescent rats.

Human studies that rely on surveys and self-reports have reported that adolescents who commonly abuse alcohol rarely report withdrawal symptoms upon cessation of drinking compared to adults (Martin and Winters, 1998). Yet, to date there has been little experimental evidence examining acute withdrawal and hangover from ethanol in adolescents. Because of ethical constraints on providing ethanol to human adolescents, animal models may prove useful when examining ethanol withdrawal during adolescence. The purpose of this study was to compare the anxiogenic component of ethanol withdrawal in both adolescent and adult rats so we might further understand the factors contributing to ethanol abuse during the adolescent period.

2. General methods

2.1. Subjects

A total of 130 adolescent (postnatal day [P]33–35) and adult (P70–75) male Sprague–Dawley rats bred in our colony were used in these experiments. On the day after birth, litters were culled to 8–10 pups, with 6 animals of one sex and 4 animals of the other being retained whenever possible. Pups were housed with their parents in standard clear plastic breeder tubs with pine shavings until the time of weaning. Male offspring were weaned at P21, housed in same-sex littermate pairs in wire hanging cages, and maintained in a temperature-controlled vivarium on a 14:10-h light:dark cycle (lights on 0700 h) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water; female offspring were used in other projects. At all times, rats used in these experiments were maintained and treated in accordance with guidelines for animal care established by the National Institutes of Health (1986).

2.2. Drug challenge

Animals received either an intraperitoneal (i.p.) injection of a 20% ethanol solution (vol/vol; diluted with a 0.9% saline) at a dose of 4 g/kg or an isovolumetric saline injection, both warmed to 32 °C. Each animal in the littermate pair received the same drug challenge. After injection, each pair was placed in a solid-bottom breeder tub with pine shavings and ad-libitum access to food and water until the time of isolation before testing.

2.3. Apparatus

The adult EPM consisted of two open arms, 48.26 × 12.7 cm, and two closed arms, 48.26 × 12.7 × 29.21 cm. The adolescent EPM consisted of 30 × 8.89 cm open arms and 30 × 8.89 × 20.32 cm closed arms. These dimensions were proportionately sized based on crown–rump length and confirmed by gait analysis. Because intoxicated animals lose some of their motor coordination, small plastic edges (0.6 cm adolescents and 1.3 cm adults) were added along each side and end of the open arms to prevent subjects from falling during testing (Fernandez and File, 1996). Gaps of 4.0 cm (adolescent) and 4.5 cm (adult) at the junctions of the open

and closed arms provided easy access below the plane of the maze to allow for protected head dips over the sides of the maze. Both mazes were elevated to a height of 50 cm. A white noise generator was used to attenuate superfluous sounds during testing. All sessions were conducted under dim light (3 lx) without the experimenter present in the room and were videotaped by a camera mounted above the apparatus at a height of 147 cm. After each animal, the apparatus was cleaned with a 3% hydrogen peroxide solution and dried before the next animal was placed on the apparatus.

2.4. Procedure

At varying times following the challenge with ethanol or saline (see specific experiments), both animals in each pair were socially isolated for 30 min before testing on the EPM. Previous research has suggested that exposure to a novel environment before plus maze testing increases activity during the EPM test session (Pellow et al., 1985). During the isolation period, one animal of the pair was placed in a novel solid-bottom breeder tub with clean pine shavings, while the other animal was restrained in a plastic flat-bottom restrainer (6.35 cm diameter × 15.24 cm length for adolescents and 8.57 cm diameter × 21.59 cm length for adults; Braintree Scientific, Braintree, MA) that was placed in the home cage. Restraint was included as a manipulation before testing in the EPM given previous work suggesting that pretest stressors, including restraint (Albonetti and Farabollini, 1992), may elevate baseline levels of anxiety in the plus maze (for review and references, see Hogg, 1996; Rodgers and Dalvi, 1997), and hence might serve to potentiate mild withdrawal-related anxiogenic effects.

At the start of the EPM session, each subject was placed into the center platform facing a closed arm and its behavior on the maze was videotaped for 5 min. Behavioral measures later scored from the videotapes included open arm time, open arm entries, protected and unprotected head dips, protected and unprotected stretched attends, number of closed arm entries, and number of rears. An animal was considered to have entered an arm when all four paws were placed in the arm. An animal was considered to have exited an arm when at least two front paws were out of the arm. Protected head dips included dipping the head over the sides of the maze while in the center platform or a closed arm, while dips were considered unprotected when the animal dipped its head over the sides of the maze when on the open arm. Protected stretched attends were defined as when the animal's two hind feet remained in a closed arm or the center platform while the animal elongated its head and shoulders onto the open arm, followed by subsequent retraction. An unprotected stretched attend was defined as the same behavior, but when the animal was on the open platform.

Percentage of time spent on the open arms and percentage of open arm entries have repeatedly been shown to be reliable measures of anxiety on the EPM (Lal et al., 1991; Pellow et al., 1985). More recently, percent protected head dips and percent protected stretched attend postures have been suggested to be even more sensitive measures of anxiety, based on ethological analysis and pharmacological manipulations (Espejo, 1997; Rodgers and Dalvi, 1997; Rodgers et al., 1994). Closed arm entries and number of rears were considered indices of activity (Cruz et al., 1994; Rodgers and Dalvi, 1997).

2.5. Data analysis

Behavior was analyzed by continuous observation of videotape records and was scored by an experimenter blind to the treatment of the subjects. Behaviors on the EPM, as previously described, were compared across test conditions using between-groups analysis of variance (ANOVA) procedures, with post hoc comparisons made with Tukey–Kramer tests ($P \leq .05$).

3. Experiment 1

Previous studies have shown that following a period of chronic ethanol exposure, adult rats demonstrate increased levels of anxiety on the EPM, indexed by a lower percentage of open arm entries, less time spent on the open arms and greater percentages of protected head dips and protected stretched attend postures (File et al., 1993; Lal et al., 1991; Rodgers and Dalvi, 1997). Given that adolescent rats have been shown to be hyposensitive to many of ethanol's effects, the main objective of this experiment was to determine if adolescent rats are also hyposensitive to acute withdrawal experienced after a binge dose of ethanol, compared to adults.

3.1. Methods

A total of 60 animals were tested across the eight experimental conditions defined by the 2 (age) × 2 (drug challenge) × 2 (pretest condition) factorial design. Eighteen hours following challenge with ethanol or saline, using the procedure described in Section 2, adolescent and adult rats were restrained and/or socially isolated for 30 min and then tested on the EPM.

3.2. Results and discussion

As seen in Fig. 1, adult, but not adolescent, ethanol-preexposed rats demonstrated an increase in anxiety manifested by significant reductions in percent open arm entries and percent open arm time as well as increases in the percentage of protected head dips and stretched attend postures compared to saline controls. Significant age by drug challenge interactions were evident for percent open arm entries: $F(1,52) = 4.618$, $P < .05$; percent open arm time: $F(1,52) = 4.428$, $P < .05$; percent protected head dips:

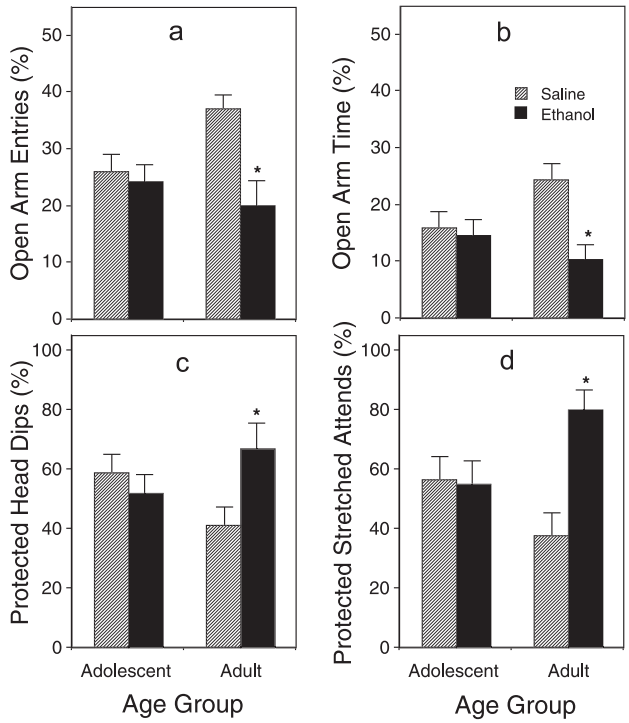

Fig. 1. Behavioral responses on the EPM of adolescent and adult rats 18 h after drug challenge: (a) percentage of open arm entries; (b) percent open arm time; (c) percent protected head dips; (d) percent protected stretched attends. Bars represent the means for each group and the vertical lines represent the standard errors of the means. Asterisks denote significant differences from saline controls with $P<.05$.

$F(1,52)=6.022$, $P<.05$; and percent protected stretched attend postures: $F(1,52)=8.112$, $P<.01$.

There were main effects of age and drug exposure for both number of rears [$F(1,52)=17.601$, $P<.0001$; $F(1,52)=8.875$, $P<.01$] and number of closed arm entries [$F(1,52)=13.421$, $P<.01$; $F(1,52)=14.295$, $P<.01$], but no interaction of these two variables (see Fig. 2). Animals preexposed to ethanol displayed fewer closed arm entries and rears than animals preexposed to saline, and adults exhibited fewer closed arm entries and rears than did adolescents.

There were no effects of pretest condition (restraint vs. no restraint) on any of the behavioral measures on the EPM, indicating that a mild restraint stress of 30 min did not facilitate manifestations of acute withdrawal at either age relative to a comparable period of isolation. Although Albonetti and Farabollini (1992) found that restraint stress increased anxiogenic behavior in the plus maze, Falter et al. (1992) reported that a wide variety of exogenous pretest manipulations did not influence any behavioral responses on the EPM. Given that the present study used socially isolated animals as the comparator group to assess the effects of restraint, it is possible that 30 min of social deprivation alone was sufficiently stressful to obscure any further impact of the restraint.

The reduction in percentage of open arm time and entries and the increases in percentage of protected head dips and stretched attends seen in adult ethanol-preexposed animals are all indices of an increase in anxiety, an anxiogenic profile during acute ethanol withdrawal that confirms previously reported results (File et al., 1993; Lal et al., 1991). However, 18 h after a single large dose of ethanol, adolescent animals did not exhibit any signs of elevated anxiety that would be indicative of an acute withdrawal reaction. Both adolescent and adult ethanol preexposed animals, however, displayed a reduction in locomotor activity, measured by number of rears and closed arm entries. This decrease in activity could reflect some indication of acute withdrawal among adolescents, given previous research reporting reductions in locomotor activity during acute ethanol withdrawal in adult animals (File et al., 1993; Lal et al., 1991).

4. Experiment 2

In Experiment 1, adolescent rats demonstrated little evidence of the acute ethanol withdrawal that was evident in adult rats 18 h after challenge with a large dose of ethanol. Previous studies have shown that adolescents sometimes eliminate ethanol from their system faster than adults following administration of the same gram per kilogram doses (Brasser and Spear, 2002; Little et al., 1996). Due to potential age differences in elimination times, it was possible that adolescent animals may not have experienced acute withdrawal because ethanol had been eliminated from their systems for a longer amount of time pretest than for adults.

The main objective of Experiment 2 was to determine if anxiogenic symptoms of acute ethanol withdrawal would be evident in adolescent animals when tested at the same time postclearance as adult animals in Experiment 1. To accomplish this, clearance times were first determined in a group of adult and adolescent animals in Experiment 2a. Based on these results, a separate group of adolescent animals were tested at equivalent postclearance times as the adults in

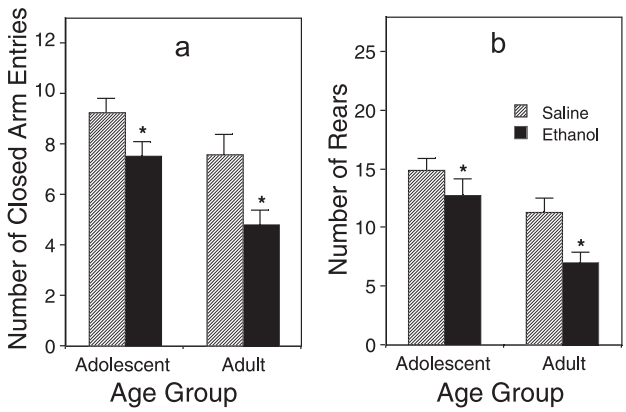

Fig. 2. Number of closed arm entries (a) and number of rears (b) in the EPM 18 h after drug challenge. Asterisks (*) indicate significant differences from saline control animals on data collapsed across age ($P<.05$). There was also a main effect of age, but no interaction with drug challenge, with adolescents showing significantly more of both behaviors than adults.

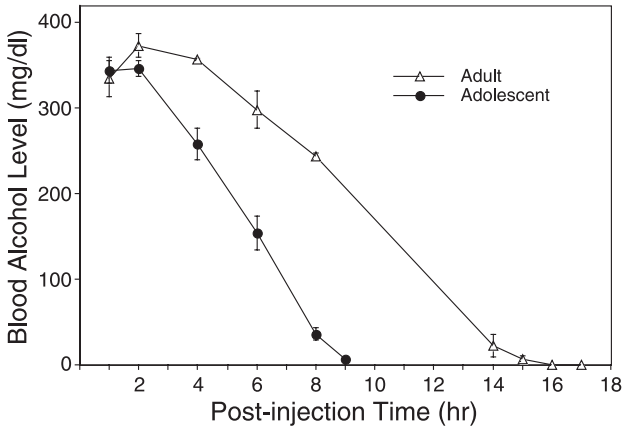

Fig. 3. Blood alcohol levels of adolescent and adult rats at different times following challenge with 4 g/kg intraperitoneal ethanol. Vertical lines denote the standard errors of the means for each group at each time point.

Experiment 1 to test the hypothesis that adolescents may exhibit adult-typical anxiogenic effects when tested at comparable times postelimination.

4.1. Methods 2a

Time for ethanol clearance was determined in a separate set of male rats ($N=46$) using a between-subjects design with a 4-g/kg ethanol challenge. Blood alcohol levels were measured at 1, 2, 4, 6, 8, and 9 h for adolescent animals and at 1, 2, 4, 6, 8, 14, 15, 16, and 17 h for adult animals. Blood samples were obtained from the tail vein, centrifuged, and the plasma collected and stored at -80 °C until being analyzed in 5-μl aliquots using an ANALOX (AM-1) analyzer. The oxidation of ethanol to acetaldehyde in the presence of alcohol oxidase allows the Analox instrument to calculate the ethanol concentration in a sample by measuring the maximum rate of oxygen consumption, which is proportional to the concentration of ethanol. A 100-mg/dl standard was used to calibrate the instrument, with the instrument being recalibrated every 10 samples to ensure an accuracy of ±5 mg/dl.

4.2. Results and discussion 2a

The BALs measured at both 1 and 2 h after ethanol administration did not differ significantly between adolescent and adult animals. As seen in Fig. 3, adolescent animals cleared ethanol from their systems at approximately 9 h, whereas adults reached undetectable BALs at approximately 15 h. Thus, when animals were tested 18 h postadministration in Experiment 1, adults were tested approximately 3 h after clearance, while adolescents were examined about 9 h following elimination of the ethanol challenge. To assess whether the longer delay between ethanol clearance and testing of the adolescents could have disrupted detection of withdrawal effects in the animals in Experiment 1, adolescents were tested at 3 h after clearance (i.e., 12 h postinjection) in the following experiment.

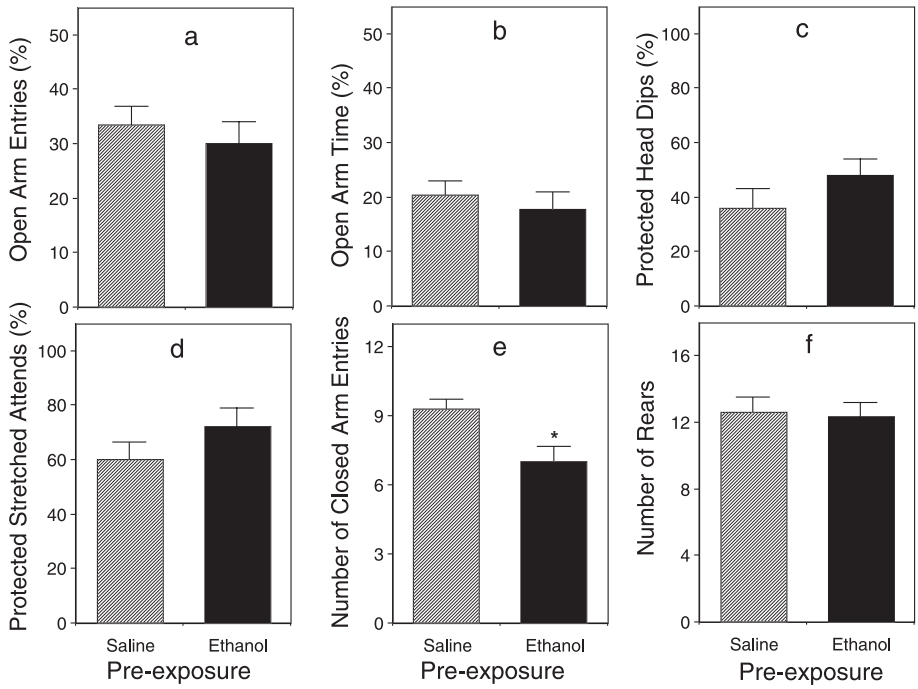

Fig. 4. Behavioral measures in the EPM of adolescent rats tested 12 h after drug challenge: (a) percent open arm entries; (b) percent open arm time; (c) percent protected head dips; (d) percent protected stretched attend postures; (e) number of rears; and (f) number of closed are entries. Asterisks denote significant differences from saline controls with $P<.05$.

4.3. Methods 2b

A total of 24 adolescent animals were tested across the four experimental conditions defined by the 2 (drug challenge) ×2 (pretest condition) factorial design. Adolescent animals were restrained and/or socially isolated 12 h following challenge with ethanol or saline and then immediately tested on the EPM for a 5-min session using the procedures outlined in Section 2.

4.4. Results and discussion 2b

The results of Experiment 2b are shown in Fig. 4. As in Experiment 1, there were no significant effects of pretest condition on any of the behavioral measures, so the results shown are collapsed across this variable. Adolescent animals tested 3 h after ethanol clearance (i.e., 12 h after drug administration) showed no signs of acute withdrawal-induced anxiety in the EPM, with no significant effect of drug challenge on the measure of percentage of open arm entries, percentage of open arm time, percentage of protected head dips and percentage of protected stretched attend postures. However, as seen in Fig. 4e, adolescents preexposed to ethanol did exhibit significantly fewer closed arm entries compared to saline control animals, $F(1,20) = 7.967$, $P < .05$. There was no significant effect of drug challenge, however, on rearing (Fig. 4f).

Even when tested at the same time after ethanol clearance as adults, adolescent animals still failed to demonstrate signs of increased anxiety during acute ethanol withdrawal. As in Experiment 1, however, adolescent animals preexposed to ethanol did display a reduction in activity during the acute withdrawal testing period. To the extent that a suppression of activity could be interpreted as a sign of acute withdrawal, the decrease in the number of closed arm entries could reflect a residual effect of prior ethanol exposure that emerges in both adult and adolescent animals.

5. General discussion

Eighteen hours following an injection of 4 g/kg ethanol ip, adults showed clear anxiogenic effects of acute withdrawal on the EPM, while adolescents did not. Following a 4-g/kg ip injection of ethanol, adolescents cleared ethanol from their system faster than adults, so the failure to find anxiogenic withdrawal effects in adolescence may have been due to a difference in clearance times. Yet, after being tested at the same time postclearance as adults (i.e., 12 h postadministration), adolescents still failed to demonstrate any anxiogenic signs of acute withdrawal on the EPM. Both adults and adolescent animals did, however, demonstrate a significant reduction in overall activity (as measured by closed arm entries on the EPM) when tested at the same time postclearance.

In the adults, anxiogenic effects of acute ethanol withdrawal were evident in terms of a reduction in percent of open arm entries and open arm time, with concurrent increases in percent of protected head dips and stretched attend postures. These results add to the growing body of literature that continues to verify the EPM as a sensitive behavioral test for detecting changes in anxiety (Espejo, 1997; File, 1994; File et al., 1991, 1993; Gatch et al., 1999; Lal et al., 1991; Pellow et al., 1985; Rodgers and Dalvi, 1997; Rodgers et al., 1994). Whereas previous studies reporting anxiogenic responses on the EPM during ethanol withdrawal have done so following chronic exposure to ethanol (File, 1994; File et al., 1991, 1992, 1993; Gatch et al., 1999; Lal et al., 1991), to our knowledge, the present study is the first to demonstrate increases in anxiety in the EPM during acute ethanol withdrawal from a single large dose of ethanol. These findings extend other results that have reported an increase in anxiety indicative of a hangover state in laboratory animals using behavioral tests such as conditioned place aversion (Morse et al., 2000; but see also Gauvin et al., 1997) and the operant PTZ drug discrimination paradigm (Gauvin et al., 1992, 1993).

In contrast to the data from adults, adolescents were found to be insensitive to increases in anxiety during the hangover phase following acute ethanol administration, at least when indexed on the EPM. One could argue, however, that a failure to find withdrawal-induced anxiety in adolescent animals may stem from initially elevated anxiety levels demonstrated by saline-treated adolescent controls, relative to their adult counterparts. This explanation seems unlikely, however, given that a similar lack of anxiogenic effects during acute withdrawal has been recently observed in adolescent animals (Varlinskaya and Spear, 2002b) in a test situation (a social interaction test) where no age-associated differences in initial anxiety levels were observed (Varlinskaya and Spear, 2002a). In that study, whereas adult male rats were found to be more anxious during ethanol withdrawal as indexed by an overall decrease in social interactions, adolescent animals actually showed a facilitation of social interactions during withdrawal. Taken together, the results suggest that even when using different behavioral tests to examine anxiety, adolescents fail to exhibit the withdrawal-induced anxiety that is seen in adults. These findings add to the list of ethanol effects for which adolescent animals appear to be less sensitive than adults, findings that include not only ethanol's sedative, motor impairing, and anxiolytic effects (Little et al., 1996; Silveri and Spear, 1998; Varlinskaya and Spear, 2002a; White et al., 2002), but also anxiogenic effects seen during ethanol hangover.

It is not the case, however, that adolescents show no detectable alterations on the EPM during the withdrawal period. Both the adolescent and adult ethanol preexposed animals demonstrated a significant decrease in locomotor activity during withdrawal as indexed via number of closed arm entries. Significant hypoactivity in conjunction with

anxiogenic effects on the EPM previously have been reported in adult rats during the withdrawal period following chronic ethanol treatment (File, 1994; File et al., 1993; Gatch et al., 1999; Lal et al., 1991). The results of the present study suggest that this withdrawal-induced hypoactivity is also evident during acute withdrawal and is a form of ethanol withdrawal/hangover that may be apparent in both adolescent and adult animals.

Consistent with the developmental dissociation of the two withdrawal-induced behaviors of hypoactivity and anxiety, there is evidence that these two measures of acute withdrawal on the EPM are modulated by different neural systems, with pharmacological manipulations that reduce withdrawal-related anxiogenesis having little or no effect on the hypoactivity commonly observed (File et al., 1992; Gatch et al., 1999; Gauvin et al., 1993; Jung et al., 2000; Lal et al., 1991). Although the neurochemical systems contributing to withdrawal-related hypoactivity are still unknown, attenuated GABAergic systems, increases in NMDA activity, and alterations in the serotonergic system have been implicated in the withdrawal-induced anxiety commonly observed following ethanol exposure (for references and review, see Gatch and Lal, 2001; Valenzuela, 1997).

Age-specific alterations in these neural systems could contribute to the insensitivity of adolescents to the anxiogenic component of withdrawal/hangover. Research indicates that GABA brain concentrations are lower in adolescent versus adult rats (Coyle and Enna, 1976), and their receptor subunit composition differs from the more mature pattern (Fritschy et al., 1994; Laurie et al., 1992), with these receptors also reported to be hyposensitive during adolescence (Moy et al., 1998). Developmental overexpression of the NMDA receptor system also could contribute to the lack of withdrawal-associated anxiogenesis during adolescence, with NMDA subtype binding peaking during the third postnatal week and declining thereafter into adulthood (Pruss, 1993). Ontogenetic changes in the serotonergic system have also been reported (Darmani et al., 1996; Dinopoulos et al., 1997), although studies focused on this neurotransmitter are limited during the adolescent transition. Whether developmental attenuation in these or other neural systems contributes to the lack of ethanol-withdrawal-induced anxiety behaviors among adolescents is an area for future investigation.

The results of this study may have important implications for adolescent alcohol consumption in humans. Recent results of the Monitoring the Future Study, 2000, have demonstrated that adolescents frequently reported binge drinking (Johnston et al., 2001). Because the negative consequences of alcohol consumption have been suggested as cues to moderate consumption on subsequent occasions, an insensitivity to ethanol-induced anxiogenesis during withdrawal/hangover could limit the amount of negative feedback adolescents receive. Clinical self-report data have suggested that human adolescents report a lower incidence of hangover following ethanol consumption than do adults (Martin and Winters, 1998). To the extent that a similar effect can be found in humans as in this study, an attenuated sensitivity to adverse anxiogenic consequences during the withdrawal phase following a binge drinking episode could serve as a permissive factor to support further binge drinking.

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