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ETHANOL-INDUCED CONDITIONED TASTE AVERSION IN MALE SPRAGUE DAWLEY RATS: IMPACT OF AGE AND STRESS

Rachel I. Anderson, M.S., **Elena I. Varlinskaya, Ph.D.**, and **Linda P. Spear, Ph.D.**
Center for Development and Behavioral Neuroscience, Department of Psychology, Binghamton University, Binghamton, New York 13902-6000

Abstract

Background—Age-specific characteristics may contribute to the elevation in ethanol intake commonly reported among adolescents compared to adults. The present study was designed to examine age-related differences in sensitivity to ethanol's aversive properties using a conditioned taste aversion (CTA) procedure with sucrose serving as the conditioned stimulus. Given that ontogenetic differences in responsiveness to stressors have been previously reported, the role of stressor exposure on the development of CTA was also assessed.

Methods—Experiment 1 examined the influence of 5 days of prior restraint stress exposure on the expression of CTA in a 2-bottle test following 1 pairing of a sucrose solution with ethanol. In Experiment 2, the effects of 7 days of social isolation on the development of CTA were observed using a 1-bottle test following multiple sucrose-ethanol pairings.

Results—The present study revealed age-related differences in the development of ethanol-induced CTA. In Experiment 1, adolescents required a higher dose of ethanol than adults to demonstrate an aversion. In Experiment 2, adolescents required not only a higher ethanol dose but also more pairings of ethanol with the sucrose conditioned stimulus. No effects of prior stressor exposure were observed in either experiment.

Conclusions—Together, these experiments demonstrate an adolescent-specific insensitivity to the aversive properties of ethanol that elicit CTA, a pattern not influenced by repeated restraint stress or housing in social isolation. This age-related insensitivity to the dysphoric effects of ethanol is consistent with other work from our laboratory, adding further to the evidence that adolescent rats are less susceptible to negative consequences of ethanol that may serve as cues to curb consumption.

Keywords

adolescent; rat; ethanol; conditioned taste aversion; stress exposure

Adolescence is a developmental period characterized by considerable transformations that are highly conserved across species. In addition to neural and hormonal changes, increases in social activity, novelty seeking, impulsivity and risk-taking are frequently reported during this transitional phase (see Spear, 2000; 2010). In line with these behavioral changes, alcohol use is commonly initiated during adolescence. Survey results from the 2008 Monitoring the Future study indicate that as many as 72% of high school seniors have tried alcohol at least once and that nearly 30% have been drunk in the past 30 days (Johnston et al., 2009).

Address correspondence to: Linda P. Spear, Department of Psychology, Binghamton University PO Box 6000, Binghamton, NY 13902-6000, lspear@binghamton.edu, Phone: (607) 777-2825, Fax: (607) 777-6418.

Elevated ethanol intake is characteristic of rodent models of adolescence as well, using a conservative age range (postnatal days [P] 28 to 42) to typify the developmental period during which adolescent-characteristic neural and behavioral features are evident in both males and females (Spear, 2000). Adolescent rats often voluntarily consume 2–3 times more ethanol than do adults (Brunell and Spear, 2005; Doremus et al., 2005; Vetter et al., 2007). For this reason, animal models of adolescence are particularly useful in examining factors that may contribute to the age-related elevation of ethanol consumption, given ethical constraints limiting this line of research in human subjects.

There are several age-specific characteristics that may lead to elevated ethanol consumption during adolescence. For example, adolescents appear to be more sensitive to the social-facilitating effects of ethanol (Varlinskaya and Spear, 2002) and less sensitive than adults to many of the aversive consequences of ethanol that may serve as cues to curb intake, such as motor impairment (Hollstedt et al., 1980; Silveri and Spear, 2001; White et al., 2002), suppression of locomotor activity (Little et al., 1996), social impairment (Varlinskaya and Spear, 2002), and sedation (Draski et al., 2001; Moy et al., 1998; Silveri and Spear, 1998; 1999; 2002; 2004). Additionally, adolescent rats experience attenuated anxiogenesis compared to adults following acute ethanol withdrawal (Doremus et al., 2003; Varlinskaya and Spear, 2004). Together these age-related differences in sensitivity to various effects of ethanol may permit or even encourage higher ethanol consumption by adolescents relative to adults.

Conditioned taste aversion (CTA) is one method used to assess the dysphoric effects of alcohol and other drugs. This procedure involves the ingestion of a novel-flavored solution (conditioned stimulus; CS) paired with the effects of a particular drug (unconditioned stimulus; US). When given a subsequent opportunity to consume the drug-paired flavor, the degree to which an animal avoids the solution provides an index of the relative dysphoria experienced following the previous encounter with the drug. Supporting this interpretation, Green and Grahame (2008) report that CTA negatively correlates with ethanol intake across a wide variety of strains and selected lines of rats and mice, suggesting that low sensitivity to the aversive properties of ethanol may facilitate high ethanol intake. A recent study in our laboratory (Vetter-O'Hagen et al., 2009) demonstrated an age-related insensitivity to ethanol-induced taste aversion using saccharin as a CS, with both male and female adolescents requiring higher doses of ethanol than their adult counterparts to elicit a CTA. This initial study exploring the role of social context in sensitivity to the dysphonic effects of ethanol reported that adolescent males (but not females) who were exposed to an unfamiliar social partner for 24 hr following the pairing of ethanol with a saccharin solution CS demonstrated an attenuated sensitivity to the aversive properties of ethanol relative to males that remained isolated for this period. While the previously reported reduction in sensitivity to ethanol's effect in the presence of a partner among adolescent males was interpreted as an effect of social experience during intoxication (Vetter-O'Hagen et al., 2009), we cannot preclude that exposure to an unfamiliar conspecific may have been stressful for a test subject. Therefore, the decreases in sensitivity to the aversive properties of ethanol seen in that CTA paradigm might have been stress-induced.

Human adolescents are confronted with a number of social and environmental challenges that are potentially stressful (Buchanan et al., 1992; Spear, 2000), and drinking to cope with problems is associated with extensive alcohol use in adolescence (Cooper et al., 2000). Research conducted in laboratory animals, although still limited, has revealed age-related differences in responsiveness to stressors, with adolescent rodents sometimes displaying increased sensitivity to stressors when indexed via hormonal responses as well as behavioral alterations (Brunell and Spear, 2005; Doremus-Fitzwater et al., 2009; Romeo et al., 2006; Stone and Quartermain, 1997). Given these ontogenetic differences in stress responses, it is

possible that exposure to stressors might differentially impact sensitivity to the aversive effects of ethanol in adolescents compared to adults. Some experimental evidence suggests that exposure to stressors either immediately before or after ingestion of the CS may attenuate CTA (Misanin et al., 2006; Bourne et al., 1991; Revusky & Reilly, 1989), likely via an interruption in the learned association between the CS and US. However, no attempts have been made to assess age-related differences in stress-induced alterations using ethanol-induced CTA. Therefore, the effects of two different stressors on ethanol-induced CTA were examined in adolescent and adult animals in the present experiments: Repeated restraint (Experiment 1) and isolate-housing (Experiment 2).

An additional explanation for the elevated ethanol consumption commonly reported during adolescence involves possible age-related differences in hedonic sensitivity. In general, consummatory behaviors toward appetitive stimuli (such as food and other natural rewards) increase during adolescence. This change may be due to alterations in the hedonic value of the stimuli; that is, individuals at this age may consume more of a particular reinforcer either because they find it more rewarding or because they find it less rewarding and must consume more in order to achieve the positive consequences. If adolescents do indeed exhibit a partial anhedonia, they may seek out more natural and drug rewards to compensate for this age-related insensitivity to natural reinforcers. Sucrose consumption and preference are measures commonly used as a presumptive index of anhedonia (Katz, 1982; Konkle et al., 2003; Matthews et al., 1995; Willner et al., 1987), given evidence that animals subjected to chronic mild stress (an animal model of depression) typically reduce their intake of sweetened solutions (Gronli et al., 2004; Willner et al., 1987). There is some evidence that sucrose intake is generally more affected by prior stress exposure than that of saccharin (Harris et al., 1998; Grønli et al., 2005). In order to allow for assessment of hedonic sensitivity at both ages, sucrose, but not saccharin (Vetter-O'Hagen et al., 2009) was chosen as the CS in the present study, with the baseline sucrose consumption (prior to ethanol exposure) serving as an index of relative of stress-induced anhedonia.

Given that our previous study revealed no sex differences in ethanol-induced CTA in adult animals and decreased sensitivity to the aversive properties of ethanol when intoxication occurred in the presence of the peer in adolescent male but not female rats (Vetter-O'Hagen et al., 2009), only male subjects were used in the present experiments.

EXPERIMENT 1: The influence of repeated stressor exposure on ethanol-induced conditioned taste aversion in adolescent and adult rats

Adolescents are less sensitive than adults to many of the aversive consequences of ethanol that may serve as cues to curb intake. Because adolescence may be a particularly stressful developmental period (Arnett, 1999) and because stress may interact with ethanol's effects (see Pohorecky, 1981; 1990), this experiment was designed to assess the influence of stressor exposure on ethanol's negative effects when indexed via CTA in both stressed and non-stressed adolescent and adult rats. Restraint stress was chosen as a stressor since it is primarily psychological in nature and does not involve physical pain or harm to the animal (Herman and Cullinan, 1997; Weinberg et al., 2007). Furthermore, this stressor has been shown to produce sobering effects in adolescent and adult rats (Doremus-Fitzwater et al., 2007).

Subjects

A total of 172 male Sprague-Dawley rats bred in our colony at Binghamton University were used in Experiment 1. On postnatal day (P) 1, litters were culled to eight to 10 pups, with a ratio of six males to four females maintained whenever possible. Subjects were weaned on

P21 and housed in pairs with a same-sex littermate unless otherwise specified. Animals were maintained in a temperature-controlled vivarium on a 14:10-hr light:dark cycle (lights on at 7 AM) and were at all times treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee. Subjects had ad libitum access to food (Purina lab chow, Lowell, MA) and water prior to experimentation. Eight to nine animals were assigned to each of the 20 experimental conditions defined by the 2 (age: adolescents, adults) × 2 (stress condition: no manipulation, restraint) × 5 (ethanol dose: 0, 0.75, 1.0, 1.5, 2.25 g/kg) factorial design. No more than one animal per litter was assigned to a given test group and all testing occurred between the hours of 10AM and 3PM.

Procedure

Subjects were re-housed with a same-age non-littermate on either P21 (adolescents) or P65–67 (adults). Seven days thereafter, subjects in the restraint stress condition were placed in restraint tubes for 90 min in a novel holding cage (a standard acrylic tub with no bedding) every day for experimental days 1–5. Restraint tubes (Tailveiner, Braintree Scientific, Braintree, MA) were Plexiglas cylinders measuring 18 × 4.7 cm for adolescents and 23 × 8.0 cm for adults (length × diameter). Each cylinder had a slot across the top and a sliding stopper that allowed for adjustment of the tube's length in order to appropriately restrain animals of varying sizes.

While subjects in the stress condition were in the restraint tubes, non-stressed animals remained in their home cages and were not manipulated. Following stressor exposure on experimental day 5, all pairs of subjects were 50% water-deprived. For this calculation, water intake for each cage over the previous 24 hr was measured and divided in half. This amount was prepared in a 100-ml bottle in addition to an extra 5 ml of water to account for fluid in the bottle inaccessible to subjects. At no time following this water deprivation procedure did animal body weights drop below 85% of age-appropriate free-feeding weights.

On experimental day 6, animals in each housing pair were separated by a mesh divider for 30 min prior to conditioning. For conditioning, each subject was given access to a single bottle containing a 10% sucrose solution for 30 min. The mesh divider allowed for measurement of individual consumption while avoiding any potential effects of social isolation (Hall, 1998). Immediately following the 30-min sucrose access period, each subject was given an i.p. injection of 0 (0.9% saline), 0.75, 1.5 or 2.25 g/kg ethanol (18.9% v/v in a 0.9% saline solution). Injection volume (rather than ethanol concentration) was adjusted to deliver varying doses (Linakis and Cunningham, 1979). Saline control animals were injected with a volume equivalent to the volume of the highest ethanol dose, and all solutions were administered at room temperature. Animals in each housing pair were assigned to the same experimental condition (i.e., animals housed together always received the same dose of ethanol or saline and were assigned to the same stressor condition).

The mesh divider was removed approximately 15 min post-injection, and a freshly-filled water bottle returned to the cage for 24 hr. On experimental day 7, fluid intake of each pair over the preceding 24 hr period (including sucrose solution consumed during conditioning) was again measured, with 50% of this volume given to each pair over the 24 hr period prior to preference testing. On test day, animals in each housing pair were again separated by a mesh divider for 30 min prior to the 2-bottle choice test. During the 1-hr test, each subject had access to two bottles: one containing a 10% sucrose solution and the other containing water.

Data Analysis

Percent body weight gain across the stressor exposure period (calculated by the formula [(post-stress weight – pre-stress weight)/(pre-stress weight)]*100), g/kg sucrose intake during conditioning and percent sucrose preference on the test day served as the dependent variables of interest. Sucrose preference scores were determined using the formula [(sucrose consumption during test)/(total fluid consumption during test)]*100; thus, sucrose intake scores higher than 50% reflected greater intake of sucrose than water, while scores lower than 50% reflected relative avoidance of the sucrose solution. Body weight gain and sucrose intake during conditioning were analyzed using 2 (age) × 2 (stress) factorial analyses of variance (ANOVAs). Percent sucrose intake on the test day was analyzed using a 2 (age) × 2 (stress) × 5 (EtOH dose) factorial ANOVA, with Dunnett's post-hoc tests used to explore main effects and interactions of interest. Subjects who did not consume any sucrose on the conditioning day were eliminated from the study.

Results

Percent Body Weight Gain—As expected, adolescents gained more weight than adults [main effect of age, $F(1,118) = 2247$, $p < 0.01$]. Additionally, there was a significant main effect of stress, $F(1,118) = 95$, $p < 0.01$, with both stressed adolescents and stressed adults gaining less weight than their non-stressed counterparts (see Figure 1).

Sucrose Intake during Conditioning—As shown in Figure 2 (left panel), adolescents consumed significantly more sucrose solution per kg body weight on the conditioning day than did adults [main effect of age, $F(1,168) = 120$, $p < 0.01$]. However, there was no impact of stress condition on intake during conditioning at either age.

Percent Sucrose Intake on Test Day—Analysis of sucrose consumption relative to total intake on the test day revealed a significant age × dose interaction [$F(4,152) = 3$, $p < 0.02$]. Saline-treated animals at both ages demonstrated relatively high sucrose preferences. Dunnett's post-hoc tests revealed significant reductions in sucrose consumption relative to the saline-injected controls following ethanol doses of 1.0 g/kg and higher in adults, but only at ethanol doses of 1.5 g/kg and higher among adolescents (see Figure 3). Prior stressor exposure did not influence sucrose preference in animals of either age.

EXPERIMENT 2: The influence of isolation stress on the development of ethanol-induced conditioned taste aversion in adolescent and adult rats

Previous results from our laboratory have indicated that isolate-housing can influence home cage ethanol consumption in adults, with isolated adults consuming less ethanol than their pair-housed counterparts—an effect that did not reach significance in adolescents (Doremus et al., 2005). The influence of post-weaning housing conditions on ethanol-induced conditioned taste aversion has been examined previously in adult female Hooded Lister rats, with no differences reported between subjects housed in a socially enriched environment or in isolation (Smith et al., 1997); however, the effects of isolate-housing on conditioned taste aversion in adolescent animals are unknown.

While conditioned taste aversion is often assessed via a two-bottle consumption test (see Exp. 1), this procedure has been modified by the Cunningham group to allow tracking of the development of the aversion across repeated pairings of ethanol using a one-bottle conditioning/test procedure (e.g., Risinger and Cunningham, 1995). This method was used in Experiment 2 to explore the influence of social deprivation on ethanol's effects by examining the development of ethanol-induced conditioned taste aversion across five conditioning days in isolate-housed and pair-housed adolescent and adult rats.

Subjects

A total of 128 adolescent and adult animals were tested across the 16 experimental conditions defined by the 2 (age: adolescents, adults) × 2 (housing condition: pair-housed, isolate-housed) × 4 (ethanol dose: 0, 0.5, 1.0, 1.5 g/kg) factorial design, with 7–8 animals placed into each experimental group.

Procedure

As in Experiment 1, animals in the pair-housed condition were placed with a same-age non-littermate assigned to the same experimental group on P21 (adolescent-tested) or P65 (adult-tested). At the same time, animals in the isolate-housed condition at each age were placed in a cage alone. Subjects were then not manipulated for an acclimation period of eight days before the onset of the experimental procedure.

On experimental day 1 (24 hr before the first conditioning day), all subjects were 50% water-deprived as described for Experiment 1. The following day, pair-housed animals were separated by a mesh divider 30 min prior to each being given access to a single bottle containing 10% sucrose for 1 hr. Following this 1-hr access period, animals were injected i.p. with one of the four doses of ethanol (0, 0.5, 1.0 or 1.5 g/kg). Each pair of animals housed together received the same ethanol dose. Approximately 15 min thereafter, mesh dividers were removed and regular water bottles were returned to each cage for 24 hr of ad libitum access, with intake during this period used to calculate the 50% fluid amount to be given over the next 24 hr. This procedure was repeated on experimental days 3–10, with every other day serving as a sucrose intake conditioning/test day. On day 10, the final intake test day, no post-test injections were given.

Data Analysis

Baseline sucrose intake was analyzed in a 2 (age) × 2 (housing) factorial ANOVA. Sucrose intake (g/kg) on each of the four test days (i.e., experimental days 3, 5, 7, and 9) was analyzed via a 2 (age) × 2 (housing) × 4 (ethanol dose) × 5 (day) repeated measures ANOVA, as was percent of baseline sucrose intake across days. Significant effects were then subjected to Tukey's post hoc tests.

The baseline sucrose intake of four subjects was more than two standard deviations less than the respective group mean. Because significant exposure to the CS is a prerequisite for the development of conditioned taste aversion, these subjects were identified as outliers and eliminated from all analyses. Three of these subjects were isolate-housed adolescents (one each from the 0.5, 1.0 and 1.5 g/kg ethanol groups) and the remaining subject was an isolate-housed adult (from the saline control group). Additionally, 11 sucrose intake data points (i.e., <2% of the total) from various conditioning days were replaced with group means because they were either two standard deviations above or below the group mean—variations likely attributable to bottle leakage or measurement error.

Results

Baseline sucrose intake—As shown in Figure 2 (right panel), housing conditions had no effect on baseline sucrose intake among adults, whereas isolate-housed adolescents consumed significantly less sucrose than their pair-housed counterparts [age × housing interaction: $F(1,120) = 6.54$, $p < 0.02$]. There was also a main effect of age [$F(1,120) = 220.8$, $p < 0.01$], with adolescents again consuming notably more sucrose than adults.

Sucrose consumption on test days—A significant age × dose × day interaction, $F(12,432) = 3.78$, $p < 0.01$, emerged during analysis of the sucrose intake on the test days

(see Figure 4). For adults, Tukey's post-hoc tests revealed significant decreases in sucrose consumption on all four test days in the 1.0 and 1.5 g/kg ethanol groups ($p < 0.05$) when compared to saline-injected controls. For adolescents, Tukey's post-hoc tests revealed significant decreases in sucrose consumption from saline control animals only on test days 2–4 and only in the 1.5 g/kg ethanol group ($p < 0.01$). No effects of housing condition were seen.

Percent of baseline across 4 test days—Given the difference in baseline sucrose consumption on day 1 across housing condition and across age, these data were also analyzed following transformation into percent of baseline [(Test day sucrose intake/ Baseline day sucrose intake)*100] prior to further analysis. As presented in Figure 5, a significant age × dose × day interaction, $F(9,324) = 4.87$, $p < 0.01$, also emerged in the analysis of these data, with Tukey's post-hoc tests revealing a generally similar pattern of findings as for the raw consumption findings, with significantly reduced consumption relative to saline controls on all four test days for adult subjects in the 1.0 and 1.5 g/kg ethanol groups, but with significant reductions occurring only on test days 3–4 in the 1.5 g/ kg ethanol group for adolescents. Tukey's post-hoc tests also confirmed the disparity between adolescents and adults at the 1.0 g/kg ethanol dose: adults showed a significant reduction from baseline sucrose intake compared to adolescents on test days 3–4. Adolescents and adults did not differ at any other dose. Again, no effects of housing condition were evident in animals of either age.

DISCUSSION

Results from the present study revealed age-related differences in the development of ethanol-induced CTA, with adolescent males requiring higher doses of ethanol than adult males to demonstrate an aversion. These results extend findings reported in an earlier study from our lab (Vetter-O'Hagen et al., 2009) across multiple CTA paradigms using different solutions as a CS. In the current study, a 10% sucrose solution served as a highly appetitive, caloric CS in two different CTA procedures. Data from the initial sucrose consumption tests revealed greater baseline g/kg sucrose intake among adolescent than adult animals in both experiments, and hence do not support the hypothesis that adolescents are less sensitive to hedonic stimuli than adults. These results are in agreement with previous taste reactivity data from our lab demonstrating greater positive oral responses to sucrose among adolescent animals relative to their adult counterparts (Wilmouth and Spear, 2009). Interestingly, using baseline sucrose consumption as an index of relative anhedonia revealed age differences in response to different types of stressors. For both adolescents and adults, prior restraint effectively reduced body weight but the stressor did not influence sucrose consumption in animals of either age. Isolation housing, however, resulted in decreased sucrose consumption among adolescent but not adult animals. No effects of prior stressor exposure on ethanol-induced CTA were observed in animals of either age.

The CTA procedure can be varied in several ways that may influence experimental outcomes. In the earlier study from our lab, the conditioning paradigm involved one pairing of ethanol with the saccharin CS followed by a one-bottle test 48 hr later. There has been some debate regarding the optimal testing conditions in CTA models: while some researchers have favored a one-bottle test (Batsell and Best, 1993), others have argued that a two-bottle test (i.e., providing the animal with a choice between the drug-paired solution and water) is a more sensitive measure (Klein et al., 1975). According to Batsell and Best (1993), the two-bottle testing method, while appropriate for revealing an aversion, is less effective for detecting differences between groups that demonstrate varying degrees of aversion. In the present study, the two-bottle test in Experiment 1 and the one-bottle test in Experiment 2 yielded similar results. Adults developed an aversion to sucrose following a

1.0 g/kg dose of ethanol under both one-bottle and two-bottle testing conditions. Likewise, adolescents demonstrated aversion at the same ethanol dose (1.5 g/kg) regardless of test procedure. The two experiments also differed in whether animals were given one (Experiment 1) or multiple (Experiment 2) pairings of the flavored solution with ethanol. The procedure used in Experiment 2, during which subjects received a total of five pairings of sucrose with ethanol, with the latter four pairings serving as both test and conditioning sessions, was found to provide a particularly sensitive assessment of the development of CTA, amplifying age-related differences. Adolescents receiving the 1.5 g/kg ethanol dose did not demonstrate the aversion to sucrose that was evident following one pairing in adults, but instead required multiple pairings of sucrose with ethanol to display CTA. Taken together with the previous report (Vetter-O'Hagen et al., 2009), the results of the present study suggest that adolescent-associated insensitivity to the aversive properties of ethanol in the CTA paradigm is a robust phenomenon that generalizes across a number of procedural differences, including housing conditions, the nature of the CS, number of CS-US pairings, and test procedures.

Neither stressor in our experiments influenced the aversive properties of ethanol in the CTA paradigm. The results of Experiment 2 are reminiscent of the findings of a previous study (Smith et al., 1997) where no effects of extensive (12-week) post-weaning isolate-housing were found on CTA to a 1.5 g/kg ethanol dose in adult female Hooded Lister rats. A number of other studies, however, have found various types of stressors to modify the discriminative and rewarding properties of ethanol. For example, in an experiment examining the influence of stressor exposure on rewarding and aversive effects of ethanol in Wistar rats using a place conditioning paradigm, Funk and colleagues (2005) found that conditioned place aversion produced by a 1.0 g/kg ethanol dose was blocked by exposure to either footshock stress or social defeat prior to ethanol injection. In a recent study, prolonged exposure to a stressor (corticosterone in the drinking water for 7 days) was reported to induce an adaptive decrease in plasma corticosterone levels in male Long-Evans rats as well as to diminish the interoceptive effects of ethanol as demonstrated in a drug discrimination task (Besheer et al., 2009). In the Vetter-O'Hagen et al. (2009) study, however, the aversive properties of ethanol were eliminated in adolescent males by a presence of a peer during an intoxication period – an exposure to an unfamiliar conspecific that might be considered stressful. It seems unlikely, however, that experimental animals perceived any amount of stress in that study, given that the presence of an unfamiliar conspecific has pronounced stress-ameliorating (i.e., social buffering) effects in rats (Cirulli et al., 1996; Kiyokawa et al., 2004).

Previous research suggests that stressor exposure can influence expression of CTA under some circumstances. Footshock, tail-pinch stress, and swim stress have all been shown to attenuate CTA to unconditioned stimuli such as lithium chloride and morphine when administered after conditioning, between the US and CS pairing, or prior to CS presentation (Misanin et al., 2006; Bourne et al., 1991; Revusky & Reilly, 1989). Such findings suggest that examination of the effects of other types of stressors on ethanol-induced CTA may have yielded different results. It is also possible that the lack of stress effects on CTA in the present experiments could be associated with the use of a sucrose CS or water deprivation. Sucrose has been reported to attenuate physiological responses to stress (Ulrich-Lai et al., 2007), perhaps dampening the perception of stressor exposure (Foster et al., 2009). The modest water deprivation schedule employed to encourage fluid consumption during testing also might have been sufficiently stressful enough to mask any additional stressor effects. It is also feasible that the stressors used may not have been perceived as stressful at one or both ages. Indeed, when stress-induced anhedonia was indexed via baseline levels of sucrose consumption, only isolate-housed adolescents consumed significantly less sucrose than pair-housed adolescents—an age effect not seen in adult animals in Experiment 2 nor at either age following restraint stress in Experiment 1. This finding should not be surprising, given

that interactions with peers are more rewarding for adolescents than for adults (Douglas et al., 2004), and deprivation of these social interactions appears especially stressful for adolescent animals relative to their more mature counterparts (see Hall, 1998). Although restraint stress was ineffective in inducing an anhedonia response to sucrose at either age in Experiment 1, this stressor was effective in reducing body weight at both ages, suggesting that the restraint stress effects on CTA are not simply due to a lack of perception of the restraint manipulation as stressful.

Attenuated expression of CTAs among adolescents is not unique to ethanol: relative to adults, adolescent animals have demonstrated a reduced susceptibility for development of CTAs to a variety of unconditioned drug stimuli, including amphetamine (Infurna and Spear, 1979), THC (Schramm-Sapyta et al., 2007), nicotine (Shram et al., 2006; Wilmouth and Spear, 2004), and cocaine (Schramm-Sapyta et al., 2006). In the latter study, adolescents showed attenuated CTA to both cocaine and lithium chloride. These data suggest that when compared to adult animals, adolescents show a general reduction in sensitivity to CTA whether the unconditioned stimulus is addictive or not, supporting the idea that adolescents may process aversive stimuli differently than more mature animals. An alternative explanation for reduced expression of CTA in adolescent subjects is that animals of this age may not be able to learn associations between the conditioned stimulus and the effects of the drug with which it was paired. This explanation seems unlikely, given that studies within the same experimental series found that, relative to adult animals, adolescents exhibited weaker aversive responses to nicotine when indexed by means of nicotine-induced CTA, but greater sensitivity to nicotine-induced conditioned place preference (Shram et al., 2006). Indeed, there is an emerging literature showing that, whereas adolescents are less sensitive than adults to the aversive properties of drug (and even non-drug) stimuli, their sensitivity to the rewarding properties of drugs such as nicotine and cocaine appears to be greater (Badanich et al., 2006; Brenhouse and Andersen, 2008; Brenhouse et al., 2008; Shram et al., 2006; Torres et al., 2008; Vastola et al., 2002; Zakharova et al., 2009a; Zakharova et al., 2009b). Furthermore, adolescents are more sensitive to the rewarding properties of social stimuli (Douglas et al., 2004) and novelty (Douglas et al., 2003).

In summary, the results of these experiments demonstrate that adolescent rats are less sensitive than their adult counterparts to the aversive properties of ethanol when indexed via CTA. This age-related pattern of insensitivity was still evident following variation in housing conditions, the nature of the CS, number of CS-US pairings, and test procedure. Consistent with other recent work from our laboratory (Vetter-O'Hagen et al., 2009), the present study provides further evidence that adolescent rats, while demonstrating elevated levels of ethanol intake relative to adults in a number of different paradigms (Brunell and Spear, 2005; Doremus et al., 2005; Smith et al., 1997; Vetter et al., 2007), are less susceptible to negative consequences of ethanol. These findings add a developmental dimension to conclusions based on genetic analyses that lower sensitivity to ethanol-induced CTA is associated with higher levels of ethanol intake across a wide variety of selectively bred lines (Broadbent et al., 2002; Chester et al., 2003; Froehlich et al., 1988; Green and Grahame, 2008; Phillips et al., 2005). Together, these approaches suggest that aversive properties of ethanol serve as important cues for modulating consumption, and support the conclusion that age-typical insensitivities to the aversive properties of ethanol likely contribute to the elevated consumption commonly reported during adolescence.

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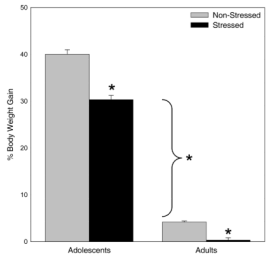

Figure 1.

Percent Body Weight Gain Before and After 5 Days of Restraint Stress. Overall, adolescents showed greater increases in body weight across the 5 days of restraint stress than adults. Stressed animals of both ages gained significantly less weight than their non-stressed counterparts.

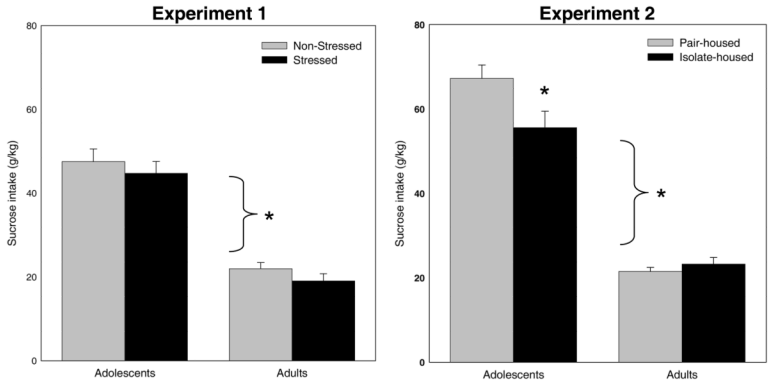

Figure 2.

Baseline Sucrose Intake. For Experiment 1, adolescents consumed more sucrose (g/kg) than their adult counterparts. No differences in sucrose intake among stressed and non-stressed animals were observed in animals of either age. For Experiment 2, adolescents again consumed more sucrose than adults. While isolate- and pair-housed adults showed similar sucrose consumption, isolate-housed adolescents consumed significantly less sucrose than pair-housed adolescents.

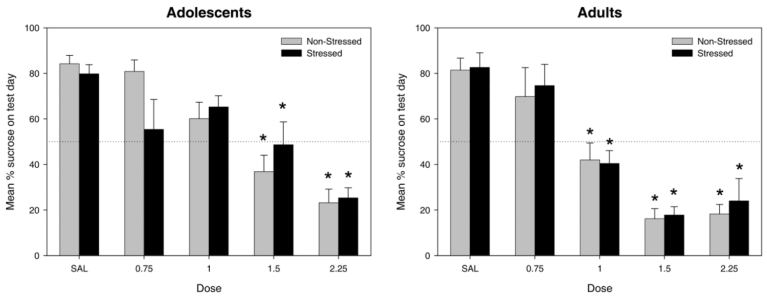

Figure 3.

Mean Percent Sucrose on Test Day. Adolescent subjects injected with ethanol doses of 1.5 g/kg or higher demonstrated a significant reduction compared to saline controls in percent sucrose consumed on the test day. Adult subjects injected with ethanol doses of 1.0 g/kg or higher exhibited an attenuation of percent sucrose intake on the test day.

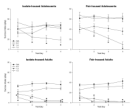

Figure 4.
Sucrose Intake Across Test Days. During the test days following the first conditioning session, adolescent animals in both housing conditions significantly reduced their sucrose intake compared to saline-injected controls following a 1.5 g/kg dose of ethanol on test days 2–4. Adult animals demonstrated a reduction in sucrose consumption on test days 1–4 following ethanol doses of 1.0 g/kg or higher.

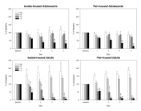

Figure 5.
Percent of Baseline Sucrose Intake Across Test Days. Across the four test days following the first conditioning session, adolescent animals in both housing conditions consumed a significantly smaller percent of their baseline sucrose intake compared to saline-injected controls on test days 3–4 following an ethanol dose of 1.5 g/kg. Adult animals, however, demonstrated a significant decrease from baseline sucrose consumption on all four test days following ethanol doses of 1.0 g/kg and higher. Adults showed a significant reduction from baseline sucrose intake compared to adolescents on test days 3–4 following conditioning with the 1.0 g/kg ethanol dose (* indicates significant difference from saline controls; + indicates significant decrease relative to adolescents).