Adolescent rats fail to demonstrate a LiCl-induced pre-exposure effect: Implications for the balance of drug reward and aversion in adolescence

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Abstract Adolescents display weaker taste avoidance induced by both abused and non-abused drugs than adults. Drug history attenuates avoidance learning in adults (the drug pre-exposure effect), but little is known about this phenomenon in adolescents. Given that the weaker taste avoidance in adolescence is thought to be a function of their relative insensitivity to the drug's aversive effects, it might be expected that the drug pre-exposure effect would be weaker in adolescents given that for some drugs this effect is mediated by associative blocking that depends on the association of environmental cues with the drug's aversive effects. To address this, in the present studies male adolescent (Experiment 1) and adult (Experiment 2) rats were given five spaced injections of LiCl prior to subsequent taste avoidance conditioning with LiCl. Consistent with past reports, adolescents displayed weaker taste avoidance than adults. While adults displayed attenuated LiCl-induced taste avoidance following LiCl pre-exposure, adolescents showed no evidence of this pre-exposure. This work is consistent with the view that adolescents are relatively insensitive to the aversive effects of drugs, an insensitivity potentially important in subsequent intake of drugs of

abuse given that such intake is a function of the balance of their rewarding and aversive effects.

Keywords Adolescent · Adult · US Pre-exposure · LiCl · Taste avoidance

Introduction

If consumption of a novel solution is followed by radiation, the rat rapidly comes to avoid its consumption on a subsequent exposure, i.e., it acquires a conditioned taste avoidance (CTA; see Garcia et al., 1955; see also www.CTAlearning.com). Although initially demonstrated with X-irradiation, such learning has been demonstrated with a wide variety of drugs ranging from classic emetics such as lithium chloride (LiCl) and apomorphine (Garcia et al., 1966) to drugs of abuse such as amphetamine (Booth et al., 1977) and morphine (Farber et al., 1976). Although taste avoidance learning is generally robust and rapidly acquired, it is affected by a variety of subject characteristics (age: Anderson et al., 2010; Schramm-Sapota et al., 2009; sex: Carroll et al., 2004; Vetter-O'Hagen et al., 2009; strain: Davis & Riley, 2007; Kosten et al., 1994) and experimental manipulations (light/dark cycle, stress: Gomez et al., 2000; Sperling et al., 2010, route of administration and drug dose: Ferrari et al., 1991).

One factor that has been extensively examined in the context of CTA is drug history. In one of the initial demonstrations of the effects of drug history on taste avoidance learning, Elkins (1974) reported that animals exposed to the synthetic antineoplastic compound cyclophosphamide prior to taste avoidance conditioning (with cyclophosphamide) displayed significantly attenuated avoidance relative to non-pre-exposed animals conditioned with the drug. Further, the magnitude of the pre-exposure effect was dependent upon the

Key Points • Adolescents and adults both displayed LiCl-induced taste avoidance.

- Adolescents displayed weaker taste avoidance than adults.
- LiCl pre-exposure attenuated taste avoidance in adults.
- LiCl pre-exposure had no effect on taste avoidance in adolescents.
- Adolescents are relatively insensitive to a drug's aversive effects.

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number of injections given during pre-exposure. Subsequent to this initial demonstration, a host of compounds such as amphetamine (Cappell & LeBlanc, 1971), morphine (Domjan & Siegel, 1983), cocaine (Glowa & Williams, 1992), ethanol (June et al., 1992), and LiCl (Braveman, 1975, 1978, 1979) have been reported to attenuate taste avoidance when given prior to conditioning with these same drugs, although animals without the drug history display robust suppression of consumption (for reviews, see Randich & LoLordo, 1979; Riley & Simpson, 2001).

Although the attenuating effects of drug pre-exposure on taste avoidance learning have been assessed with a variety of drugs and under a host of parametric conditions, one feature that is constant among the studies is the use of adult animals. This is of interest given that there are age-dependent differences in taste avoidance conditioning with a number of compounds (Doremus-Fitzwater et al., 2010; Merluzzi et al., 2014; Schramm-Sapyta et al., 2009). For example, adolescent rats consistently display weaker drug-induced CTA compared to adults when conditioned with a number of different compounds such as amphetamine (Infurna & Spear, 1979), cocaine (Schramm-Sapyta et al., 2006), ethanol (Schramm-Sapyta et al., 2010; Vetter-O'Hagen et al., 2009), morphine (Hurwitz et al., 2014), and nicotine (Wilmouth and Spear, 2004; Shram et al., 2006), among others (Anderson et al., 2010; Anderson et al., 2014; Flax et al., 2015; Merluzzi et al., 2014; Schramm-Sapyta et al., 2007; Wetzel et al., 2014). The relatively weaker taste avoidance reported in adolescents has been suggested to be a function of their relative insensitivity to the drug's aversive effects (Schramm-Sapyta et al., 2014), i.e., the drug is less aversive in adolescent subjects and thus induces a weaker avoidance response.

This explanation may be particularly relevant to the US pre-exposure effect, given that the attenuation in taste avoidance conditioning following drug pre-exposure has been argued for some drugs, e.g., the emetic LiCl, to be a function of the association of various environmental or injection cues with the aversive effects of the drug (Braveman, 1979; Cole et al., 1996; Dacanay & Riley, 1982; Davis et al., 2010; De Brugada

et al., 2003a, b, 2004, 2005; Willner, 1978; for reviews and alternative interpretations for other drugs, see Randich & LoLordo, 1979; Riley & Simpson, 2001). That is, during drug pre-exposure the injection-related cues are paired with the drug's aversive effects and when taste avoidance conditioning is subsequently attempted in the presence of these stimuli, the association between the taste and the aversive effects is blocked. If adolescents are less sensitive to these aversive effects, less conditioning should occur during drug pre-exposure and consequently blocking would be less likely to occur in this age group (relative to adults). These specific predictions were tested in the following experiment in which adolescents and adults were pre-exposed to LiCl (for five total injections) and subsequently given a novel saccharin solution followed by LiCl during taste avoidance conditioning (see Table 1).

General materials and methods

Subjects and housing

Forty-two experimentally naïve, male Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) arrived at the laboratory between post-natal days 21 and 23 (PND 21–23). Upon arrival, subjects were group housed in Opti-Rat Plus polycarbonate bins (38.9 × 56.9 × 26.2 cm; 1,181 sq. cm; 3–4 per bin) and placed on 12:12 light-dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C. Half of the subjects were assigned to Experiment 1 (adolescent assessment); the remaining half were assigned to Experiment 2 (adult assessment). Throughout each study, subjects were weighed daily for the duration of the experiment. During CTA habituation, conditioning and testing procedures (see below), subjects were temporarily transferred to individual hanging wire-mesh test cages (24 × 19 × 18 cm) located in the subjects' housing room. Minimal use of cages with wire-mesh flooring is recommended (National Research Council, 2011) due to possible foot lesions, which occur with extended

Table 1 Summary of the predicted results

Age Group	Pre-exposure Group	Conditioning Group	Prediction
Adolescents	Vehicle	Vehicle	Control consumption
		LiCl	Weak taste avoidance
	LiCl	Vehicle	Control consumption
		LiCl	Weak effects of US pre-exposure
Adults	Vehicle	Vehicle	Control consumption
		LiCl	Robust taste avoidance
	LiCl	Vehicle	Control consumption
		LiCl	Robust effects of US pre-exposure

housing in such conditions (Peace et al. 2001). Therefore, the use of wire-mesh test cages was restricted to experimental procedures and subjects were returned to their group-housed bins every day when testing was complete. All procedures occurred during the light phase (0800 h–2000 h), and unless otherwise stated, food and water were available ad libitum. The study was approved by the Institutional Animal Care and Use Committee of American University and followed the National Research Council's Guide for the Care and Use of Laboratory Subjects (2011) and the Guidelines for The Care and Use of Mammals in Neuroscience and Behavioral Research (2004).

Drugs and solutions

LiCl was dissolved in sterile isotonic saline (0.9 %) at a concentration of 0.15 M (6.4 mg/ml) and passed through a 0.2- μ m filter to remove any potential contaminants before being administered intraperitoneally (I.P.) at a dose of 0.56 mEq/kg (23.9 mg/kg). This single dose was chosen based upon previously published dose-response assessments of LiCl-induced taste avoidance that reported intermediate avoidance in adult rats at this dose (see Dacanay et al., 1984; Gore-Langton et al., 2015; Nachman & Ashe, 1973). Sodium saccharin (Sigma Aldrich) was prepared at 1 g/L in tap water for a 0.1 % (w/v) saccharin solution.

Procedure

Experiment 1: Adolescents

Drug pre-exposure and habituation Subjects in Experiment 1 were randomly assigned to a pre-exposure condition [0.56 mEq LiCl ($n = 11$) or equivolume vehicle ($n = 10$)]. On PND 25, they were weighed at 1000 h, given their first pre-exposure injection at 1500 h in an adjacent room and returned immediately to their home cages. From PND 26–28, subjects were weighed at 1000 h each day but received no injections. On PND 29, a second pre-exposure injection was given followed by 3 recovery days. This 4-day cycle continued until five total pre-exposure injections had been given.

Conditioned taste avoidance Abbreviated CTA procedures designed to maintain proper growth rates in adolescent subjects were utilized as previously published (Hurwitz et al., 2014; Wetzell et al., 2014). On PND 36 at 1000 h, water was restricted for 24 h. On PND 37, subjects were placed in individual hanging wire-mesh test cages at 1000 h where they had access to water for 45 min. Following the 45-min water access during these habituation sessions, subjects were immediately returned to their home cages with ad libitum access to water until the following day at 1000 h. This 2-day habituation cycle was repeated until the first CTA conditioning trial on

PND 45. Specifically, subjects were deprived of water on PND 44 and a novel saccharin solution was presented instead of water for 45 min on PND 45. Immediately after the session, subjects in each pre-exposure condition were rank ordered on saccharin consumption and assigned to one of two groups, such that mean saccharin consumption among groups was comparable, and injected with either vehicle or LiCl. This resulted in a total of four groups (i.e., V0, V0.56, L0, and L0.56), where the letter indicates the injection given during pre-exposure and the number the dose of LiCl administered during conditioning. Following the injection, subjects were given ad libitum water access in their home bins for 23 h and 15 min. This 2-day procedure was repeated three times for a total of four conditioning trials (CTA 4 on PND 51). On PND 53, the day following the final water deprivation session, subjects were given 45-min access to both saccharin and water in a two-bottle avoidance test with no subsequent injections. In this two-bottle test, one bottle was presented (saccharin or water) on either the left or the right side of the hanging wire test cage. As soon as the subject sampled from the first bottle, it was removed and the second bottle was presented on the opposite side. After the second bottle was sampled, it was removed and both bottles were then placed simultaneously on their respective sides of the cage. The order of presentation and side placement were counterbalanced within groups, and consumption of both saccharin and water was recorded. Following these procedures, animals were returned to their home cages with ad libitum water access. For the adolescent experimental timeline, see Fig. 1a.

Experiment 2: Adults

All experimental parameters for adults were the same as in the adolescent preparation with the exception of the timing of the specific manipulations. For the adult experimental timeline, see Fig. 1b.

Data analysis: Adolescents and adults

To determine whether LiCl pre-exposure significantly affected adolescent or adult growth, body weights were analyzed over pre-exposure using two separate 2×5 mixed model ANOVAs, each with between-subjects factors of Pre-exposure Drug (VEH, LiCl) and within-subjects factors of Day (Adolescents: PND: 25, 29, 33, 37, 41; Adults: PND: 88, 92, 96, 100, 104). In the event of a Pre-exposure Drug $\times$ Day interaction, differences between groups were tested with one-way ANOVAs with Bonferroni corrections.

During CTA conditioning, differences in saccharin consumption for both adolescents and adults were analyzed using two separate $2 \times 2 \times 4$ mixed model ANOVAs with between-subject factors of Pre-exposure Drug (VEH, LiCl) and Conditioning Dose (0, 0.56) and a within-subjects factors of

Fig. 1 Procedural timeline for adolescents (a) and adults (b)

Trial (1-4). In the event of a three-way interaction in either of these analyses, simple effects of Trial at each Pre-exposure Drug and Conditioning Dose (multivariate analysis) and the effects of Pre-exposure Drug at each Conditioning Dose and Trial (univariate analysis) were assessed and followed with Bonferroni-corrected multiple comparisons as warranted. For both adolescents and adults on the two-bottle CTA test, saccharin and water consumption were recorded for each subject and percentage saccharin of total fluid consumption (i.e., saccharin/saccharin + water) was analyzed with two separate 2×2 ANOVAs with factors of Pre-exposure Drug (VEH, LiCl) and Conditioning Dose (0, 0.56). Two-way interactions were followed by univariate analyses for simple effects at each level of Pre-exposure Drug and Conditioning Dose and by Bonferroni-corrected pairwise comparisons as warranted. Where indicated by appropriate interactions, differences in saccharin consumption between groups for individual trials were tested using one-way ANOVA's followed by Tukey's HSD post-hoc tests.

Results

Adolescence

Body weight Body weights were unaffected by LiCl given during the pre-exposure phase. The 2×5 mixed model ANOVA assessing whether LiCl pre-exposure affected adolescent growth indicated significant effects of Day [$F(4,16) = 1193.442$, $p < 0.001$], such that the weights of the adolescent subjects increased over the course of the pre-exposure period (see Fig. 2). There was no significant effect of Pre-exposure Drug nor was there a significant Pre-exposure Drug $\times$ Day interaction. Adolescent body weights increased an average

of 5.53 g daily during the pre-exposure period until Day 37 at which point water deprivation procedures began (see above).

Conditioned taste avoidance LiCl-conditioned subjects displayed significant taste avoidance, and there was no effect of LiCl pre-exposure. The $2 \times 2 \times 4$ mixed model ANOVA revealed significant main effects of Trial [$F(3,15) = 5.869$, $p < 0.05$] as well as a significant Trial $\times$ Conditioning Dose interaction [$F(3,15) = 22.083$, $p < 0.001$]. There were no effects of, nor significant interactions with, Pre-exposure Drug. Pairwise comparisons of Conditioning Dose and Trial revealed that (collapsed across Pre-exposure Drug) Group 0.56 consumed significantly less saccharin than Group 0 on Trial 2 [$F(1,17) =$

Fig. 2 Mean ($\pm$ SEM) animal weights by pre-exposure group for the pre-exposure phase of the study. LiCl pre-exposure days are designated by vertical dotted lines; LiCl pre-exposure (0.56 mEq) occurred at PND 25, 29, 33, 37, and 41. The 2×5 mixed model ANOVA indicated significant effects of PND [$F(4,16) = 1193.442$, $p < 0.001$]. There was no effect of Pre-exposure Drug, nor was there a significant interaction of Pre-exposure Drug and Day. Significance for all tests was ≤ 0.05 ; $n = 10-11$ per group

Fig. 3 Mean amount of saccharin consumed over conditioning ($\pm$ SEM) (Panel a); Groups V0.56 and L0.56 consumed significantly less saccharin relative to their vehicle controls (Groups V0 and L0). This effect was independent of drug pre-exposure. Percentage saccharin consumption on the two-bottle test ($\pm$ SEM) (Panel b); Groups V0.56 and L0.56 consumed a significantly lower percentage of saccharin than Groups V0 and L0. Again, this effect was independent of drug pre-exposure. * $p < 0.05$ from vehicle conditioned groups

21.156, $p < 0.001$], Trial 3 [$F(1,17) = 63.45$, $p < 0.001$] and Trial 4 [$F(1,17) = 92.884$, $p < 0.001$] (see Fig. 3a). In relation to changes in consumption over trials (collapsed across Pre-exposure Drug), saccharin consumption for Group 0 (subjects conditioned with vehicle) did not differ from Trial 1 to Trial 2, increased on Trials 3 and 4 relative to Trial 2 and did not differ between Trials 3 and 4. Saccharin consumption for Group 0.56 significantly decreased from Trial 1 to the remaining trials, but did not differ between Trials 2, 3, and 4. The 2×2 ANOVA on percentage of saccharin consumed during the two-bottle test indicated significant effects of Conditioning Dose [$F(1,20) = 63.245$, $p < 0.001$]. There was no significant effect of Pre-exposure Drug or a significant interaction of Pre-exposure Drug and Conditioning Dose. A one-way ANOVA assessing differences in percentage of saccharin consumption on the two-bottle test revealed a significant effect [$F(1, 20) = 69.792$, $p < 0.001$], and Tukey's HSD post-hoc for the two-bottle test indicated that Groups V0.56 and L0.56 drank a significantly lower percentage of saccharin than Groups V0 and L0 (see Fig. 3b).

Adults

Body weight Body weights were unaffected by LiCl given during the pre-exposure phase. The 2×5 mixed model ANOVA assessing whether LiCl pre-exposure affected adult growth indicated significant effects of Day [$F(4,16) = 140.475$, $p < 0.001$], such that the weights of the adult subjects increased over the course of the pre-exposure period (see Fig. 4). There was no significant effect of Pre-exposure Drug nor was there a significant Pre-exposure Drug $\times$ Day interaction. Adult body weights increased an average of 1.53 g daily during the pre-exposure period until Day 100 at which point water deprivation procedures began (see above).

Conditioned taste avoidance LiCl-conditioned subjects displayed significant taste avoidance which was weakened by LiCl pre-exposure. The $2 \times 2 \times 4$ mixed model ANOVA revealed significant effects of Trial [$F(3,15) = 6.079$, $p < 0.05$] and significant interactions of Trial $\times$ Pre-exposure Drug [$F(3, 15) = 5.165$, $p < 0.05$], Trial $\times$ Conditioning Dose [$F(3,15) = 38.551$, $p < 0.001$] and Trial $\times$ Conditioning Dose $\times$ Pre-exposure Drug [$F(3,15) = 4.139$, $p < 0.05$]. A univariate analysis used to evaluate simple effects of Conditioning Dose at each Pre-exposure Drug on each Trial revealed significant differences on Trials 2, 3, and 4 for both vehicle pre-exposed [Trial 2: $F(1,17) = 61.434$; Trial 3: $F(1,17) = 77.209$; Trial 4: $F(1,17) = 283.134$] and LiCl pre-exposed [Trial 2: $F(1,17) = 15.331$; Trial 3: $F(1,17) = 14.198$; Trial 4: $F(1,17) = 57.248$] subjects. Bonferroni-corrected multiple comparisons revealed that Groups V0.56 and L0.56 drank significantly less saccharin than Groups V0 and L0 on Trials 2, 3, and 4. Further, Group V0.56 drank significantly less saccharin than

Fig. 4 Mean ($\pm$ SEM) adult weights by pre-exposure group for the pre-exposure phase of the study. LiCl pre-exposure days are designated by vertical dotted lines; LiCl pre-exposure (0.56 mEq) occurred at PND 88, 92, 96, 100, and 104. The 2×5 ANOVA indicated significant effects of PND [$F(4,16) = 140.475$, $p < 0.001$]. There was no effect of Pre-exposure Drug, nor was there a significant interaction of Pre-exposure Drug and Day. Significance for all tests was ≤ 0.05 ; $n = 10$ –11 per group

Group L0.56 on the same trials. Groups L0 and V0 did not differ at any point in conditioning (Fig. 5a). The 2×4 ANOVA on the percentage of saccharin consumed during the two-bottle test indicated significant effects of Conditioning Dose [$F(1,20) = 323.802$, $p < 0.001$], as well as a significant interaction of Conditioning Dose $\times$ Pre-exposure Drug [$F(1,20) = 22.587$, $p < 0.001$]. There was no significant main effect of Pre-exposure drug. Tests for simple effects of Conditioning Dose at each Pre-exposure Drug indicated significant differences [Vehicle: $F(1,17) = 270.500$; LiCl: $F(1,17) = 84.007$], and multiple comparisons indicated that Groups V0.56 and L0.56 drank a significantly lower percentage of saccharin than Groups V0 and L0. In addition, Group V0.56 drank a significantly lower percentage of saccharin than Group L0.56 (Fig. 5b).

Adolescent-adult conditioned taste avoidance In an effort to compare adolescent and adult LiCl-induced avoidance directly, for each age group consumption on Trial 4 (the CTA Test)

Fig. 5 Mean amount of saccharin consumed ($\pm$ SEM) over conditioning (Panel a); Groups V0.56 and L0.56 consumed significantly less saccharin relative to their vehicle controls (Groups V0 and L0), and Group L0.56 drank more than Group V0.56. Percentage saccharin consumption ($\pm$ SEM) on the two-bottle test (Panel b); Groups V0.56 and L0.56 consumed a significantly lower percentage of saccharin than their vehicle controls (Groups V0 and L0). Further, Group L0.56 drank a greater percentage of saccharin than Group V0.56. * $p < 0.05$ from vehicle conditioned group, ^ $p < 0.05$ from LiCl pre-exposed group

for animals injected with LiCl during conditioning (Groups VL and LL) was transformed to a percentage shift from its own control, i.e., animals injected with vehicle during conditioning (Groups VV and LV). A 2×2 ANOVA revealed significant main effects of Pre-exposure [$F(1,18) = 40.794$, $p < 0.001$] and Age [$F(1,18) = 23.154$, $p < 0.001$], as well as a significant interaction of Pre-exposure and Age [$F(1,18) = 31.021$, $p < 0.001$]. Pairwise comparisons of Pre-exposure and Age on the CTA Test revealed that adults pre-exposed to vehicle and injected with LiCl during conditioning (Group Adult V0.56) displayed a significantly greater percentage shift in saccharin consumption than did adolescents pre-exposed to vehicle and conditioned with LiCl (Group Adol V0.56) [$F(1,18) = 59.276$, $p < 0.001$]. There was no difference in the percentage shift in saccharin consumption between adults and adolescents pre-exposed and conditioned with LiCl (Groups L0.56) (see Fig. 6). Adults pre-exposed to vehicle and conditioned with LiCl (Group Adult V0.56) displayed a significantly greater percentage shift in saccharin consumption than adults pre-exposed and conditioned with LiCl (Group Adult L0.56) [$F(1,18) = 71.481$, $p < 0.001$]. There was no difference in the percentage shifts in saccharin consumption for adolescents pre-exposed to vehicle or LiCl.

Discussion

The finding that in adults LiCl-induced taste avoidance was significantly attenuated by prior exposure to LiCl is consistent with earlier work assessing such effects in adults (Braveman, 1975; Riley et al., 1976; Cain & Baenninger, 1977). These attenuating effects have generally been reported to be a function of the similarity of the pre-exposure and conditioning

Fig. 6 Percentage shift in saccharin consumption ($\pm$ SEM) from controls on the CTA Test (CTA Trial 4) for adolescent and adult subjects; Group Adol V0.56 displayed a significantly lower percentage shift in saccharin consumption from controls than did Group Adult V0.56. Further Adult Group V0.56 displayed a greater percent shift in consumption than did Group Adult L0.56. * $p < 0.05$ from vehicle conditioned group

environments (and procedures) and have been argued to result from the blocking of taste avoidance conditioning by stimuli that were associated with the aversive effects of LiCl during drug pre-exposure (Braveman, 1979; Cole et al., 1996; Dacanay & Riley, 1982; Davis et al., 2010; de Brugada et al., 2003a, b, 2004, 2005; Willner, 1978). In this context, it is interesting that LiCl-induced taste avoidance in the adolescent subjects was unaffected by LiCl pre-exposure. The absence of a pre-exposure effect in adolescents is consistent with the position that due to a reduced sensitivity to LiCl's aversive effects in this age group, little conditioning to environmental or injection cues occurred during pre-exposure and thus little blocking of taste avoidance conditioning was produced by these cues.

Although the premise of the present report is that adolescent insensitivity to the aversive effects of drugs weakens conditioning and in turn the likelihood of blocking (drug pre-exposure effect), there are several issues with this interpretation. First, there was no independent assessment of this relative insensitivity to the aversive effects of LiCl. The interpretation is primarily based on the well characterized differences in taste avoidance learning between adolescents and adults (see above). It will be important to provide either behavioral or neurochemical evidence of a reduced reaction to LiCl in the two age groups to substantiate the current interpretation. A second issue concerns why the aversive effects of LiCl were insufficient to establish blocking in the adolescent subjects but could nonetheless induce a taste avoidance (albeit weak). That is, it was assumed that the aversive effects were too weak in adolescents to establish environmental conditioning, but taste avoidance conditioning could still occur. Interestingly, recent work by Gore-Langton et al. (2015) has shown that the taste avoidance design is much more sensitive in detecting the aversive effects of LiCl than the place conditioning procedure, i.e., doses of LiCl sufficient to establish a taste avoidance were unable to produce a place aversion. Accordingly, blocking might not be evident in adolescents (due to the relatively weak aversive effects of the drug and the relatively weakness of environmental stimuli to become associated with LiCl's aversive effects). Although possible, it should be noted that the work of Gore-Langton was with adults, and it is not known if similar differential taste/place conditioning would be evident in adolescents.

In addition to these general issues, there are also a number of alternative explanations for the failure to see any effects of drug pre-exposure in the adolescent subjects. For example, it is possible that adolescent rats simply have deficits in learning that preclude (or weaken) the acquisition of conditioning during pre-exposure. As noted, adolescents display weaker taste avoidance conditioning in general, and this, too, could be a function of poorer learning in this group. Although possible, adolescents actually display greater drug-induced place preferences (see Badanich et al., 2006; Belluzzi et al., 2004;

Brenhouse & Andersen, 2008; Douglas et al., 2003; Torres et al., 2008; Vastola et al., 2002; Zakharaova et al., 2009), suggesting that learning in general is not impaired in adolescents. While it is possible that there is a selective learning deficit associated with aversive conditioning (including taste avoidance learning), classic fear conditioning work predicated upon contextual learning suggests that age does not influence the acquisition or extinction of conditioning (Denenberg & Kline, 1958; Kirby, 1963). Interestingly, the retention of such avoidance responses does display a U-shaped function of age (Denenberg & Kline, 1958; Kirby, 1963; Campbell & Campbell, 1962), suggesting that retention of such learning is age dependent.

The failure to see any effects of drug pre-exposure on taste avoidance learning in adolescents could also be the result of a greater motivation to consume substances with a positive hedonic value or that they have an increased sensitivity to positive consequences (Spear & Varlinskaya, 2010; Wilmouth & Spear, 2009). Specifically, if adolescents generally increase consumption of solutions with positive hedonic properties (like saccharin), such an increase might limit their ability to demonstrate a taste avoidance. If so, consumption of the LiCl-associated saccharin solution in the non-pre-exposed subjects might be impacted (increased), resulting in a generally weaker avoidance than that seen with adults. Because of this general increase in consumption, the ability to see a difference from the LiCl-pre-exposed, conditioned subjects might be affected, limiting the ability to see a LiCl pre-exposure effect. Although possible, there are several arguments against this explanation. First, the increase in consumption should not be evident only in the non-pre-exposed, conditioned animals, i.e., one would also expect to see an increase in the LiCl pre-exposed, conditioned animals, such that a difference would still be expected between the two groups. Secondly, there was no evidence among any group of adolescents that there was an increase in consumption of saccharin (relative to their own water baselines or relative to saccharin consumption in adults), suggesting that at least under the present conditions there was no increase in the hedonic value of saccharin. A 2×2 ANOVA comparing adolescent and adult consumption of water on the fourth habituation trial and of saccharin on its first exposure prior to conditioning revealed that there was no significant difference between the amount of water consumed on the fourth habituation trial and the amount of saccharin consumed on the first exposure to saccharin for either age group. Further, adolescent and adult subjects did not differ on either of these measures. Although it appears that adolescents did not differentially react to saccharin relative to water or did not differ from adults, others have reported different hedonic responses between adolescents and adults. As such, the extent to which hedonic reactivity may play a role in weaker taste avoidance or drug pre-exposure remains unknown.

In earlier work assessing age differences in taste avoidance learning, Misanin et al. (1997) pre-exposed weanling (PND 21–24), young adult (PND 84–94), and aged (PND 674–695) rats to either LiCl or vehicle once a day for 6 days. Twenty-three hours following the last pre-exposure injection, all subjects were given access to saccharin followed by LiCl or vehicle. On the day following conditioning, animals were given access to both saccharin and water in a final two-bottle test. Under these conditions, weanling pups and young adult animals displayed effects of pre-exposure (attenuated avoidance) and there were no differences between the groups in terms of the magnitude of the pre-exposure effect (see also Valliere et al., 1988). The fact that weanling pups displayed a LiCl pre-exposure effect contrasts with the present results in which no such effects were seen in adolescent subjects. Although it is possible that the attenuating effects of LiCl pre-exposure have a U-shaped function such that the attenuating effects of drug pre-exposure are produced in weanling pups and adults, while they are not produced in adolescents, there is one important difference between the present experiments and the work of Misanin and his colleagues that may account for the differential effects. Specifically, as reported here, adolescents and adults differed significantly in the rate and degree of taste avoidance (adolescents < adults). Such a difference parallels that seen with a host of other drugs in the taste avoidance preparation (see Introduction). Interestingly, in Misanin et al. (1997) there was no significant difference in LiCl-induced taste avoidance between weanling pups and young adult rats (either those pre-exposed to vehicle or to LiCl). It may be the case that differences in avoidance learning (reflective of differential sensitivity to the drug's aversive effects) are necessary for the effects of US pre-exposure to be different between the two age groups (given that a reduced sensitivity to the drug's aversive effects is what is proposed to mediate the weaker effects of LiCl pre-exposure reported here). It would remain to be determined why the different age groups have this differential sensitivity. It should also be noted that other differences between the present work and that of Misanin et al. (and Valliere et al., 1988) may account for the differences reported in the effects of LiCl pre-exposure, including the spacing of the pre-exposure injections and the interval between pre-exposure and conditioning. It is not known to what extent these procedural differences might also have influenced the effects of pre-exposure.

Subsequent to the initial submission of the current manuscript, Saalfield and Spear (2015) reported that adolescent pre-exposure to ethanol attenuated ethanol-induced taste avoidance learning in both early and late adolescence (adult comparisons were not run, and as such it is not known to what degree adolescents would differ from adults with similar pre-exposure). Although the present results contrast with those of Saalfield and Spear, there are many factors that could influence such differences, including the drug and doses used in

pre-exposure and conditioning, as well as the number and duration of drug pre-exposures. It is interesting in this context to note that the mechanism underlying the effects of drug pre-exposure in adults is dependent upon the specific drug tested along with the specific parameters noted above (for a review, see Riley & Simpson, 2001). More work with LiCl (at different doses and pre-exposure regimens) and with other drugs is needed to assess under what conditions adolescents display effects of drug pre-exposure.

The fact that adolescent subjects did not display any attenuating effects of drug history is consistent with the position that adolescent rats are relatively insensitive to the aversive effects of drugs. This relative insensitivity could have implications for the initiation and escalation of drug use in adolescents. Work from our laboratory and others suggests that the overall affective response to a drug (and its potential for use and abuse) is thought to reflect the balance between the rewarding and aversive properties of that drug (Brockwell et al., 1991; Simpson & Riley, 2005; Wise et al., 1976). Specifically, the aversive properties of drugs limit drug use and the rewarding properties maintain it, and although reward and aversion are orthogonal, such that they are dissociable and can vary independently, the aversive effects can influence the propensity to continue taking the drug (Doremus-Fitzwater et al., 2010; Riley, 2011). Given this balance, if adolescent populations are less sensitive to the aversive properties of drugs as indexed by CTA, then the potential for drug use in adolescence is higher. This evidence further substantiates adolescence as a risk period during development.

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