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Persistent expression of methamphetamine-induced CTA in periadolescent rats

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ABSTRACT

It is well documented that the transition from periadolescence to adulthood produces profound changes in motivated behavior, and furthermore, attenuates the aversive experience of abused drugs. Little is known, however, about adolescent memory for the conditioned aversive effects of abused drugs following retention intervals that span this developmental transition. The present experiment investigated methamphetamine-induced conditioned taste aversion (CTA) in periadolescent rats to determine if the magnitude of conditioning was altered following retention intervals that extend to adulthood. Rats consumed saccharin (0.1%, w/v) and were immediately injected with saline or methamphetamine (3.0 mg/kg) either once (PND 40) or three times (PND 38–40), and memory was assessed one or 50 days later on post natal days 41 or 90, respectively. Rats exhibited robust methamphetamine-induced CTA one and 50 days after conditioning, and the strength of responding did not change as a function of retention interval, regardless if animals were trained with one or three saccharin–methamphetamine pairings. These findings indicate that the expression of memory for the aversive effects of methamphetamine was resistant to degradation throughout the developmental period of periadolescence to adulthood.

1. Introduction

The developmental transition from adolescence to adulthood produces profound changes in motivated behavior (Spear, 2000; Doremus-Fitzwater et al., 2010). Maturational changes in brain motivational systems are hypothesized to increase novelty seeking and peer-directed behaviors during periadolescence (Spear, 2000; Chambers et al., 2003; Teicher et al., 1995). Interestingly, these developmental changes also produce an attenuated response to the aversive effects of abused drugs, such as amphetamine and cocaine (Infurna and Spear, 1979; Spear and Brake, 1983; Schramm-Sapyta et al., 2006) and for the purely emetic compound lithium chloride (LiCl; Misanin et al., 1983). Little is known, however, about adolescent memory for the conditioned effects of an abused drug, particularly following retention intervals that span the developmental transition from adolescence to adulthood.

Evidence from experiments investigating memory for appetitive learning indicates that adolescent rats are vulnerable to alterations in the expression of conditioned responding as a function of increasing retention intervals. For example, Li and Frantz (2009) investigated the magnitude of conditioned responding for a cocaine-conditioned light cue that was acquired in self-administration experiments conducted during adolescence or adulthood. Cue-induced responding was exhibited by both the adolescent and adult-onset groups following various withdrawal periods, e.g., retention intervals of 1, 14, 30, and 60 days. The frequency of responding for the cocaine-associated cue light, increased, or "incubated" in adult-onset rats following various retention intervals after the final self-administration session; however, the adolescent-onset group exhibited a relative attenuation of the incubated response. Although the adolescent-onset rats demonstrated cue-induced responding, it did not significantly increase following retention intervals compared to adults, thus demonstrating that post-acquisition modulation of responding was weakened in adolescent-onset rats. Moreover, Campbell et al. (1968) showed that periadolescent (~PND 29–34) and adult (PND 90+) rats learned a light–dark discrimination task, and testing after various retention intervals revealed substantial decreases in the magnitude of conditioned responding 38, 75 and 150 days later in the periadolescents, but not the adults. Together, these findings suggest that rats trained during periadolescence showed memory for conditioning after various retention intervals; however, they exhibited modulated expression of memory over long delays. The authors suggested that the post-learning alterations in responding may be related to the developmental changes in the central nervous system of periadolescent rats (Campbell et al., 1968; Campbell, 1984; Li and Frantz, 2009).

The present experiment investigated the long-term retention of methamphetamine-induced aversive conditioning in periadolescent rats by using the conditioned taste aversion (CTA) procedure. CTA is an associative process by which animals learn that a particular taste is associated with an aversive outcome, such as malaise, and subsequent experience with the taste results in avoidance of the food (see Freeman and Riley, 2009). To observe CTA in the laboratory, animals

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consume a novel solution, the conditional stimulus (CS), and afterward, they are administered an unconditional stimulus (US) that has aversive effects. A conditioned aversion is observed if animals avoid consumption of the CS on subsequent exposures. CTA learning appears to be a unique form of conditioning because the aversive, interoceptive effects produced by a particular stimulus are preferentially associated with a taste, relative to exteroceptive cues also present in the learning context, which also precede the onset of nausea (Garcia and Koelling, 1966). CTA is acquired rapidly, following a single CS–US pairing (Garcia et al., 1955), and furthermore, it is acquired, albeit to a lesser magnitude, when an interstimulus interval is imposed between the CS and US (Revusky and Garcia, 1970). Once acquired, the avoidance response appears to be remembered over long retention intervals (Dragoin et al., 1973). These features suggest that CTA adapted as a form of aversive conditioning to protect against the selection of toxic foods.

The rapid acquisition and robust nature of taste aversion learning makes the CTA procedure an excellent method to investigate memory for aversive drug effects (Meehan and Riccio, 2009). Previous research consistently shows that a CTA acquired during adulthood is expressed following long retention intervals between conditioning and testing. For example, adult rats conditioned with cyclophosphamide or amphetamine exhibited similar magnitudes of CTA when tested either 1 or 90 days after training, indicating that retention of CTA was resistant to degradation after long conditioning-to-testing delays (Carey, 1973; Dragoin et al., 1973). Interestingly, the results from studies investigating the long-term retention of CTA acquired during development have been mixed, with some experiments reporting that LiCl-induced CTA is susceptible to forgetting over delays that correspond to maturation of motivational systems and others showing robust expression following such retention intervals. For example, Guanowsky et al. (1983) reported that post-weanling (~PND 26) and adult rats acquired CTA after a single sucrose–LiCl pairing and the magnitude of the response was diminished after a 28-day retention interval in the maturing rats, but not in the adults (also see Ader and Peck, 1977; Steinert et al., 1980; Misanin et al., 1983). Other reports, however, suggest that post-weanling rats (~PND 23) administered sucrose–LiCl or chocolate milk–LiCl pairings exhibited robust CTA when tested 21 and 28 days after acquisition (Klein et al., 1977 and Kraemer et al., 1988, respectively).

To date, no experiments have assessed the long-term expression of CTA in developing animals when a drug of abuse is the US. The aforementioned literature on the retention of CTA has focused on purely emetic compounds, and it is of interest to investigate memory for learning produced by stimuli that exhibit both rewarding and aversive unconditional stimulus effects, such as methamphetamine. Determining if the expression of methamphetamine-induced aversive conditioning is attenuated or enhanced in maturing rats will provide novel information about the expression of memory for aversive learning over the course of development. Adolescents in the United States use methamphetamine for recreational purposes (Johnston et al., 2009) and the findings of the present experiment will provide preclinical information regarding long-term memory for conditioning that occurs during adolescent drug use.

Adolescent development in the rat is generally proposed to extend from approximately PND 30–60 (Spear and Brake, 1983; Spear, 2000). The designation of PND ~30–40, ~40–50, and ~50–60 as periadolescence, mid-adolescence, and late-adolescence, respectively, is used in the present manuscript (Chambers et al., 2003; Izenwasser, 2005). Rats were conditioned with methamphetamine either once (PND 40) or three times during periadolescence (PND 38–40) and were tested one (PND 41) or 50 days later during adulthood (PND 90). The retention interval and the number of conditioning trials were the factors of interest. Adult rats exhibit robust expression of methamphetamine-induced CTA (Martin and Ellinwood, 1973) and moreover, the CTA produced by amphetamine is expressed, unchanged, by adult rats over long retention intervals (Carey, 1973). Methamphetamine was chosen as the US because relative to other abused drugs, less is known about its conditioned aversive effects, and moreover, adolescent populations are abusing methamphetamine at high rates, further necessitating preclinical research on this highly addictive compound (Johnston et al., 2009). It was hypothesized that the magnitude of methamphetamine-induced avoidance behavior would decrease after long, but not short, retention intervals. This prediction was based on experiments showing that rats exhibited memory deficits after retention intervals that coincide with the maturation of motivational systems (Campbell et al., 1968; Ader and Peck, 1977; Steinert et al., 1980; Misanin et al., 1983; Guanowsky et al., 1983; Li and Frantz, 2009).

2. Methods

2.1. Animals

A total of 100 male periadolescent, Sprague–Dawley rats were used (Harlan Laboratories, Inc., Indianapolis, IN). Rats arrived at the animal care facilities with surrogate dams on PND 20, and were transferred to a colony located in the psychology department at the University of South Carolina. The litters arrived with 5 male and 5 female pups per the investigators' request. Rats were weaned and were housed four, same sex rats/cage on PND 21, and were single-caged on PND 28. CTA was assessed with one male randomly selected from each litter per experimental group (Holson and Pearce, 1992). Rodent food (Pro-Lab Rat, Mouse, Hamster Chow #3000) was provided ad lib. The colony was maintained at ~21 °C, 50%±10% relative humidity and a 12L:12D cycle with lights on at 0700 h (EST). The protocol for this research methodology was approved by the Institutional Animal Care and Use Committee at the University of South Carolina.

2.2. Experimental design and procedure

The expression of methamphetamine-induced avoidance responding was assessed following either a one or a 50-day retention interval. All rats received 23.75 h of daily water restriction, beginning on PND 35 and ending on PND 41. Animals received access to 15 min of water, administered in 100 ml graduated, glass cylinder bottles on PND 36 and 37. A saccharin solution (0.1%; w/v) was used as the CS and methamphetamine (3.0 mg/kg; sc) was the US. The dose of methamphetamine was chosen based on previous research with periadolescent rats (PND 35; Infurna and Spear, 1979). In that study, periadolescents did not readily acquire CTA following a single CS–US paring when the dose of amphetamine was 1.0 mg/kg, but did exhibit learning following conditioning with 4.0 mg/kg. Furthermore, pilot studies in our laboratory indicate that a single CS–US pairing using methamphetamine 3.0 mg/kg produces CTA in periadolescent rats.

Periadolescent male rats received saccharin–methamphetamine or saccharin–saline pairings either 1 or 3 times (1× or 3×). Rats in the METH-1×-41 (n=13), METH-1×-90 (n=13), SAL-1×-41 (n=12), SAL-1×-90 (n=12) groups received one CS–US pairing on PND 40 and were administered a two-bottle test either one (PND 41) or 50 (PND 90) days after acquisition. Animals in the 1× condition were given a 15 min access to water in the graduated cylinders on PND 38 and 39, and were injected with saline following water consumption. The METH-3×-41 (n=13), METH-3×-90 (n=13), SAL-3×-41 (n=12), SAL-3×-90 (n=12) groups were conditioned on PND 38–40 and tested on PND 41 or 90. Thus, on PND 38–40, all rats received access to saccharin or water for 15 min and were administered an injection of saline or methamphetamine within 5 min after the bottles were removed from the animals' cages. Rats received access to water for 15 min on the afternoon of PND 40.

During two-bottle testing one bottle contained water and the other bottle contained the CS. The presentation of the bottles was balanced across groups. Animals were allowed to drink either solution for 15 min. Water bottles were placed back onto the home cage 24-h after the last acquisition trial if rats were tested 50 days after training. The PND 90 test groups experienced 24-h of water restriction prior to the two-bottle retention test. Standard water bottles were placed back onto the cage following testing, and the rats were allowed to drink ad libitum.

Two dependent measures were used to assess retention of methamphetamine-induced CTA. First, the amount of saccharin consumed on each of the three conditioning days was measured to determine acquisition of CTA. Second, preference ratios, which were derived from the two bottle tests [i.e., (saccharin − water)/(saccharin + water)], were calculated to determine preference for saccharin vs. water. All rats were housed within the same colony room during the experiment, and CS–US pairings occurred between 1400 and 1800. Standard water bottles were placed back onto the rats' home cage immediately following completion of the experiment.

2.3. Data analyses

Analysis of variance (ANOVA) techniques were conducted on the acquisition and preference data. The ANOVA conducted on the acquisition data included the between-subjects factors of conditioning trials (1 or 3 CS–US pairings) and drug (saline or methamphetamine) and the within-subjects factor of day (days 1–3) . The saccharin preference test included the between-subjects factors of drug (saline or methamphetamine), and test delay (1 or 50 days). Greenhouse–Geisser ($_{G-G}$) corrections were used on repeated measures analyses of day if violations of compound symmetry were observed. An α level of 0.05 was used for all analyses.

2.4. Drugs

Methamphetamine HCl was purchased from Sigma-Aldrich Inc. (St. Louis, MO). The dose of methamphetamine (3.0 mg/kg) was based on the salt weight and was dissolved in saline. Drug solutions were prepared fresh daily.

3. Results

3.1. Acquisition

Saccharin consumption for animals administered 1× and 3× conditioning trials are shown in Fig. 1A and B, respectively. Rats in the 1× and 3× groups showed different saccharin consumption during the initial saccharin exposure. The 1× conditioning groups consumed 9.2 ± 0.16 ml (mean ± SEM), whereas the 3× conditioning groups drank 8.2 ± 0.16 ml. Rats in the 1× groups were 2 days older than those in the 3× groups during novel saccharin exposure, and also exhibited different weights. The 1× animals weighed more than the 3× rats during novel consumption, i.e., 122.1 ± 1.37 g and 117.7 ± 1.37 g, respectively. Each rat's novel saccharin consumption score was therefore divided by that animal's weight to correct for weight differences between the 1× and 3× conditioning groups. The analysis on the weight corrected data revealed that the 1× rats consumed more saccharin than the 3× animals during the novel exposure to saccharin [$F(1, 97) = 6.8$ $p < .05$]. To determine if this difference is related to different magnitudes of saccharin neophobia or general fluid intake differences, the amount of water consumed on the day prior to the first saccharin exposure (weight corrected) was analyzed. Rats in the 1× groups consumed 0.08 ml (±0.001) whereas 3× animals drank 0.07 ml (±0.001), and this difference was significant [conditioning trials: $F(1, 95) = 42.6$ $p < .001$], thus suggesting general fluid intake differences rather than different magnitudes of saccharin neophobia.

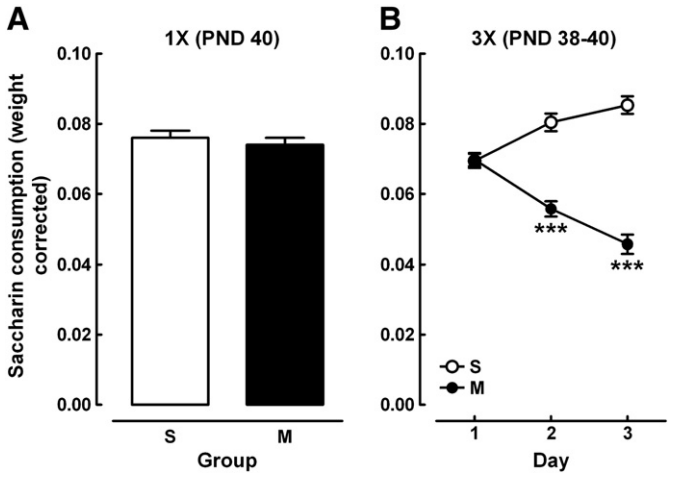

Fig. 1. Mean saccharin consumed (±SEM) during acquisition. The 1× and 3× conditioning data are shown in panels A and B, respectively, for animals injected with saline (S) or methamphetamine (M; 3.0 mg/kg, sc). *** indicates significant differences between the saline and methamphetamine groups, $p < .001$. n = 24–26/group.

The drug × day mixed factorial ANOVA (2 × 3), conducted on the weight corrected acquisition data from the 3× groups indicates that controls exhibited attenuation of neophobia, whereas rats administered methamphetamine acquired saccharin avoidance behavior over the three conditioning trials [drug: $F(1, 47) = 76.6$, $p < .001$; day × drug : $F(2, 94) = 57.2$ $p < .001$]. Comparisons between the saline and methamphetamine groups showed that animals conditioned with methamphetamine exhibited significantly less saccharin consumption than rats injected with saline on conditioning days two and three, respectively [$F(1, 47) = 56.0$, $p < .001$ and $F(1, 47) = 116.0$, $p < .001$; see Fig. 1B].

3.2. Preference tests

The saccharin preference data, from the 1 and 50 day retention tests (PND 41 and 90, respectively), are shown in Fig. 2A. Positive scores indicate a saccharin preference and negative scores describe a preference for water. The drug × conditioning trials × test delay

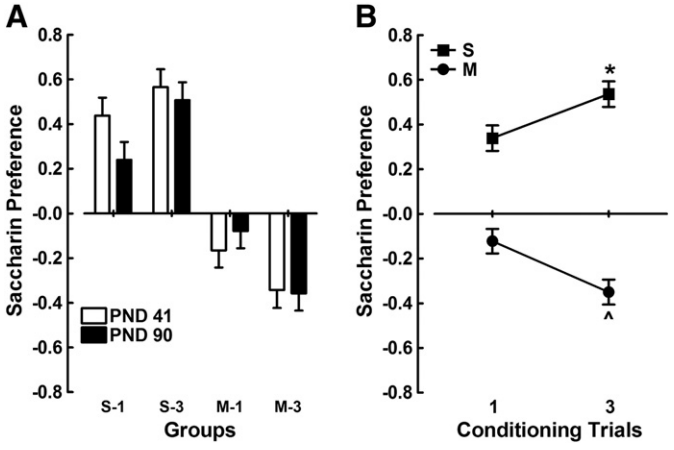

Fig. 2. (A) Mean saccharin preference ratios (±SEM) during two-bottle testing that occurred on either one (PND 41) or 50 (PND 90) days after conditioning for rats treated with saline (S) or methamphetamine (M; 3.0 mg/kg, sc). Negative scores indicate a preference for water (i.e., CTA) whereas positive scores describe a saccharin preference. (B) The mean saccharin preference ratios from the drug × conditioning trial interaction. ^ and * indicate significant differences between the M-3 and M-1 groups, $p < .05$, and between the S-3 and S-1 groups, $p < .05$, respectively. n = 24–26/group.

(2×2×2) ANOVA revealed that the methamphetamine US produced saccharin avoidance behavior [drug: $F(1, 91) = 145.1$, $p < .001$], whereas the saline groups exhibited saccharin preference. Moreover, the significant drug×conditioning trial interaction [$F(1, 91) = 14.4$, $p < .001$] indicated that animals in the METH-3× groups exhibited a greater magnitude of saccharin avoidance behavior (i.e. preference for water) than rats trained with a single conditioning trial, and that rats in the SAL-3× groups showed a greater saccharin preference than animals in the SAL-1× groups. The lack of drug×test delay and drug×conditioning trial×test delay interactions indicates that the expression of conditioned saccharin avoidance behavior was resistant to degradation following the 50-day retention interval.

The drug×conditioning trial interaction is shown in Fig. 2B. Comparison of the METH-1× and METH-3× groups indicates that animals conditioned once showed significantly less saccharin avoidance compared to the groups that received three CS–US pairings [$F(1, 44) = 5.3$, $p < .05$]. Moreover, rats in the SAL-3× groups exhibited a significantly greater saccharin preference than animals in the SAL-1× groups [$F(1, 46) = 5.2$, $p < .05$]. The 1× conditioning groups showed lower preference scores than the 3× groups because the latter demonstrated attenuation of neophobia over three consecutive acquisition days, whereas the former groups were exposed to saccharin for a second and final exposure during 2-bottle testing. The increased magnitude of saccharin preference observed in the 3× conditioning group thus indicates different magnitudes of neophobia to the CS.

Given that differences in baseline saccharin preference in the controls may have biased the statistical outcome in the two-bottle results, percent of control values for the mean saccharin preference scores were analyzed. The conditioning trials×test delay (2×2) ANOVA demonstrated that 3× conditioning trials produced more saccharin avoidance than the 1× conditioning trial manipulation [conditioning trial: $F(1, 48) = 21.7$, $p < .001$]. Neither the main effect of test nor the conditioning trial×test delay interactions were significant (data not shown).

These findings demonstrate that a single methamphetamine conditioning trial induced a weaker magnitude CTA relative to rats administered 3 CS–US pairings, and furthermore, that conditioned responding was resistant to degradation over the 50-day retention interval, regardless of the number of acquisition trials.

4. Discussion

The CTA procedure was used to assess potential changes in the magnitude of methamphetamine conditioned avoidance responding following retention intervals that correspond to the developmental period spanning periadolescence to adulthood. This is the first experiment to characterize the long-term retention of conditioned avoidance behavior produced by an abused drug in maturing animals. Although the conditioned avoidance response was expected to be observed following the 50-day retention interval, it was hypothesized that the magnitude of responding would degrade as the retention interval was increased. The present experiment demonstrated that periadolescent rats acquired robust methamphetamine-induced CTA following one or three conditioning trials, but there was no evidence that the strength of conditioned responding degraded as a function of retention interval. Rats tested 50 days after conditioning exhibited similar avoidance behavior to animals tested 24-h later. Moreover, although a single acquisition trial induced a weaker conditioned response than three conditioning trials, both treatment regimens produced consistent expression of saccharin avoidance when tested 1 or 50 days after conditioning. These results indicate that after periadolescent animals acquired CTA the response remained robust across development, and quite resistant to degradation, regardless of the strength of conditioning during acquisition.

The pattern of methamphetamine-induced CTA observed in periadolescent rats is similar to the memory for amphetamine conditioning reported in adult rats (Carey, 1973; Martin and Ellinwood, 1973). For example, Carey (1973) demonstrated that adult rats administered amphetamine (2.0 mg/kg) 30 min after, but not 30 min before, consumption of the saccharin CS exhibited stable CTA approximately 50 days after conditioning. Because adults were not tested in the present experiment, it is not known if they would have exhibited changes in the expression of methamphetamine-induced CTA over a comparable 50-day delay. One possibility is that adults trained on PND 90 and tested 50 days later would have exhibited an incubated response, or an increased magnitude of conditioned responding, relative to periadolescents tested after the same retention interval. It is unlikely that adults would have exhibited attenuated responding across the retention interval because Dragoin et al. (1973) and Carey (1973) reported that memory for cyclophosphamide and amphetamine-induced CTA was intact on daily tests that were conducted for 90 or 50 consecutive days after conditioning, respectively. The findings of the present experiment are in accord with these studies by showing that methamphetamine-induced CTA is robust after a 50 day retention interval.

The excellent retention of conditioning observed in the present experiment is in marked contrast to experiments which show that the developmental transition to adulthood produces a degradation of responding for appetitive conditioning procedures, such as those discussed in the introduction. Thus, although this developmental period produced attenuated responding for a secondary reinforcer (Li and Frantz, 2009) and mediated forgetting of a light/dark discrimination (Campbell et al., 1968) task relative to adults, for example, the interval spanning adolescence and adulthood did not result in an altered expression of methamphetamine-induced CTA. Fundamental differences in the nature of conditioning between CTA and appetitive learning may account for some of the relative differences in vulnerability to developmental alterations in conditioned responding reported by the present research and the Li and Frantz (2009) and Campbell et al. (1968) studies. CTA appears to be a unique form of learning by which organisms avoid taste stimuli that are associated with toxic and perhaps fatal outcomes (Garcia and Koelling, 1966). As mentioned previously, one of the special features of CTA is that it is acquired rapidly and it is robust, and that was clearly demonstrated in the present experiment by conditioning periadolescent rats with a single CS–US pairing. In the Li and Frantz (2009) and Campbell et al. (1968) studies, however, the rats acquired appetitive conditioning after multiple training episodes. Moreover, conditioning in these tasks involves the association of exteroceptive stimuli such as cue lights and light/dark gradients with an appetitive outcome, respectively, and these types of stimulus relations are known to be vulnerable to forgetting, and thus alterations in conditioned responding (Perkins and Weyant, 1958; Riccio et al., 1994). The stable memory for methamphetamine-induced CTA may be less susceptible to degradation over the developmental transition from adolescence to adulthood when compared to appetitive conditioning studies because of the robust nature of taste conditioning.

It is interesting that the memory produced by one methamphetamine conditioning trial was not subject to forgetting given that a number of studies suggest that the expression of LiCl-induced CTA produced by a single CS–US pairing in post-weanling rats underwent degradation over 28 or 60 day retention intervals (Steinert et al., 1980 and Guanowsky et al., 1983, respectively). The discrepancy between the present results and those that report forgetting of LiCl-induced CTA may be related to differences in the US properties of LiCl and methamphetamine. Riley and colleagues have pointed out that the effect of a purely emetic drug like LiCl may be in marked contrast to the more complex stimulus properties produced by an abused, psychostimulant drug like methamphetamine (Busse et al., 2005; Riley et al., 2009), which is capable of supporting both avoidance and

approach learning (Wise et al., 1976; Reicher and Holman, 1977). Thus, the increased complexity and duration of action for methamphetamine, relative to LiCl, may have produced CS–US attributes and/or associations that are less likely to be forgotten over long retention intervals (Gordon and Spear, 1973; see Spear and Riccio, 1994). Although speculative, one example of how methamphetamine's increased complexity may influence CTA expression relative to that produced by LiCl is that amphetamines are known to enhance learning and memory of aversive conditioning (Martinez et al., 1980; Lee and Ma, 1995; Fenu and Di Chiara, 2003; Blaiss and Janak, 2006; Wiig et al., 2009). Thus, it is possible that methamphetamine enhanced the memory of aversive CS–US attributes, and therefore, facilitated strong retention of CTA relative to that observed with a non-amphetamine-based drug like LiCl that was used in studies reporting a decreased magnitude of responding following retention intervals in maturing rats (Steinert et al., 1980; Guanowsky et al., 1983). Overall, the present results are more similar to the findings reporting that memory for LiCl-induced CTA is intact after retention intervals that correspond to the maturation of motivational systems (Klein et al., 1977; Kraemer et al., 1988).

Regarding experimental design, it should be noted that the levels of water restriction were not equal between animals prior to two-bottle testing in the present experiment. All animals were restricted for the two days prior to conditioning (PND 35–37) and for the three days that CS–US pairings were conducted (PND 38 to 40). Rats tested 1 day after conditioning (PND 41) remained water restricted for an extra day, whereas the animals tested 50 days after conditioning received water ad libitum after the final conditioning trial on PND 40. The rats tested 50 days after conditioning were water restricted for 24-h prior to testing, which began on PND 89. Thus, although all rats received 7 days of total water restriction overall, the animals tested on PND 41 had 6 days of restriction prior to testing and those assessed 50 days later (PND 90) were water deprived for 1 day prior to testing. A differential level of water restriction was necessary during the testing phases because all rats had to be equally restricted during the acquisition phase of the experiment, and in order to assess memory for CTA 1 day after training, the animals in the immediate testing groups had to remain water restricted. If the animals tested 50 days after conditioning were restricted for 6 days prior to testing, then those animals would have experienced a total of 12 days of water restriction compared to the 6 days of restriction for animals tested 1 day after conditioning. Thus, with the current design, the levels of water restriction had to be asymmetrical. We chose to limit the total restriction period for the 50-day delay groups by using 24-h of water restriction in order decrease the overall amount of time that animals were without water.

Developmental changes, such as the remodeling of brain motivational systems that occur throughout periadolescence, mid-adolescence, and late-adolescence, have been suggested to profoundly influence motivated behavior (Spear and Brake, 1983; Spear, 2000). Previous investigation into the long-term retention of aversive and appetitive conditioning during this developmental transition suggests that maturing animals are vulnerable to forgetting (Campbell et al., 1968; Ader and Peck, 1977; Steinert et al., 1980; Guanowsky et al., 1983; Misanin et al., 1983; Li and Frantz, 2009). The present experiment reports that memory for the aversive effects of methamphetamine are not altered when assessed during periadolescence and adulthood, indicating that maturation did not influence the expression of aversive taste conditioning. The present research findings, together with those of previous studies that investigated adults indicate that memory for the aversive effects of the amphetamines are stable over long retention intervals. Future work should determine if other factors contribute to changes in the expression of CTA in developing animals. For example, it is of interest to determine if other drugs of abuse produce persistent memory for CTA, or whether retention of avoidance learning is diminished during the transition to adulthood. Such experiments should include an emetic compound such as LiCl to further investigate the parameters under which maturational changes in motivational systems alter the expression of CTA. These findings will be informative for models of adolescent development that address maturational changes in motivated behavior and drug conditioning that occurs during adolescent substance abuse.

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References

Ader R, Peck JH. Early learning and retention of a conditioned taste aversion. Dev Psychobiol 1977;10(3):213–8.
Blaiss CA, Janak PH. Post-training and post-reactivation administration of amphetamine enhances morphine conditioned place preference. Behav Brain Res 2006;10(171(2)):329–37.
Busse GD, Freeman KB, Riley AL. The interaction of sex and route of drug administration in cocaine-induced conditioned taste aversions. Pharmacol Biochem Behav 2005;81(4):814–20.
Campbell BA. Reflections on the ontogeny of learning and memory. In: Kail R, Spear NE, editors. New Jersey: Lawrence Erlbaum Associates; 1984. p. 23–38.
Campbell BA, Jaynes J, Misanin JR. Retention of a light-dark discrimination in rats of different ages. J Comp Physiol Psychol 1968;66(2):467–72.
Carey RJ. Long-term aversion to a saccharin solution induced by repeated amphetamine injections. Pharmacol Biochem Behav 1973;3:265–70.
Chambers RA, Taylor JR, Potenza MN. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. Am J Psychiatry 2003;160(6):1041–52.
Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. Brain Cogn 2010;72(1):114–23.
Dragoin W, Hughes G, Devine M, Bentley J. Long-term retention of conditioned taste aversions: effects of gustatory interference. Psychol Rep 1973;33(2):511–4.
Fenu S, Di Chiara G. Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D1 receptors. Eur J Neurosci 2003;18(7):2025–30.
Freeman KB, Riley AL. The origins of conditioned taste aversion learning: a historical analysis. In: Reilly S, Schactman TR, editors. Conditioned taste aversion: behavioral and neural processes. New York: Oxford University Press; 2009. p. 9-36.
Garcia J, Kimeldorf DJ, Koelling RA. Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science 1955:157–8.
Garcia J, Koelling RA. Relation of cue to consequence in avoidance learning. Psychon Sci 1966:123–4.
Gordon WC, Spear NE. The effects of strychnine on recently acquired and reactivated passive avoidance memories. Physiol Behav 1973;10(6):1071–5 Jun.
Guanowsky V, Misanin JR, Riccio DC. Retention of conditioned taste aversion in weanling, adult, and old-age rats. Behav Neural Biol 1983;37(1):173–8.
Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol Teratol 1992;14(3):221–8.
Infurna RN, Spear LP. Developmental changes in amphetamine-induced taste aversions. Pharmacol Biochem Behav 1979;11(1):31–5.
Izenwasser S. Differential effects of psychoactive drugs in adolescents and adults. Crit Rev Neurobiol 2005;17(2):51–67.
Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the future national results on adolescent drug use: overview of key findings, 2008 (NIH Publication No. 09-7401). Bethesda, MD: National Institute on Drug Abuse; 2009.
Klein SB, Mikulta PJ, Domato GC, Hallstead C. Retention of internal experiences in juvenile and adult rats. Physio Psychol 1977;5(1):63–6.
Kraemer PJ, Lariviere NA, Spear NE. Increase in retention of a taste aversion by weanling rats after a long interval. Anim Learn Behav 1988;16(2):191–4.
Lee EH, Ma YL. Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. Brain Res Bull 1995;37(4):411–6.
Li C, Frantz KJ. Attenuated incubation of cocaine seeking in male rats trained to self-administer cocaine during periadolescence. Psychopharmacol (Berl) 2009;204(4):725–33 Jul.
Martin JC, Ellinwood Jr EH. Conditioned aversion to a preferred solution following methamphetamine injections. Psychopharmacologia 1973;29(3):253–61.
Martinez Jr JL, Jensen RA, Messing RB, Vasquez BJ, Soumireu-Mourat B, Geddes D, et al. Central and peripheral actions of amphetamine on memory storage. Brain Res 1980;182(1):157–66.

Meehan SM, Riccio DC. Memory phenomena and conditioned taste aversion. In: Reilly S, Schactman TR, editors. Conditioned taste aversion: behavioral and neural processes. New York: Oxford University Press; 2009. p. 114–33.
Misanin JR, Guanowsky V, Riccio DC. The effect of CS-preexposure on conditioned taste aversion in young and adult rats. Physiol Behav 1983;30(6):859–62.
Perkins CC, Weyant RG. The interval between training and test trials as a determiner of the slope of generalization gradients. J Comp Physiol Psychol 1958;51(5):596–600.
Reicher MA, Holman EW. Location preference and flavor aversion reinforced by amphetamine in rats. Anim Learn Behav 1977;5(4):343–6.
Revusky S, Garcia J. Learned associations over long delays. In: Bower G, Spence J, editors. New York: Academic Press; 1970. p. 1-84. Vol 4.
Riccio DC, Rabinowitz VC, Axelrod S. Memory. When less is more. Am Psychol 1994;49 (11):917–26.
Riley AL, Davis CM, Roma PG. Strain differences in taste aversion learning. In: Reilly S, Schactman TR, editors. Conditioned taste aversion: behavioral and neural processes. New York: Oxford University Press; 2009. p. 226–61.
Schramm-Sapyta NL, Morris RW, Kuhn CM. Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacol Biochem Behav 2006;84(2):344–52.
Spear NE, Riccio DC. Memory: phenomena and principles. Boston: Allyn and Bacon; 1994.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;6(2):83-109.
Steinert PA, Infurna RN, Spear NE. Long-term retention of a conditioned taste aversion in preweanling and adult rats. Anim Learn Behav Aug 1980;8(3):375–81.
Teicher MH, Andersen SL, Hostetter Jr JC. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Brain Res Dev Brain Res 1995;89(2):167–72.
Wiig KA, Whitlock JR, Epstein MH, Carpenter RL, Bear MF. The levo enantiomer of amphetamine increases memory consolidation and gene expression in the hippocampus without producing locomotor stimulation. Neurobiol Learn Mem 2009;92(1):106–13.
Wise RA, Yokel RA, DeWit H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science 1976;191(4233): 1273–5.