Age-Dependent Differences in Morphine-Induced Taste Aversions

ABSTRACT: Adolescence is a developmental period of particular importance given the host of neurobiological changes that occur during this stage of development. Drug use and abuse is said to be a function of the balance of its rewarding and aversive effects, and any age-dependent differences in morphine's aversive effects could impact drug intake. The present experiments examined the ability of morphine sulfate (0, 3.2, 10, and 18 mg/kg) to induce taste aversions in adolescent and adult rats under high (20-min fluid access each day; Experiment 1A/B) and low (50% of ad libitum access; Experiment 2A/B) deprivation conditions. In both studies, adolescent and adult rats were given a novel saccharin solution to drink and were subsequently injected with morphine. Independent of the deprivation condition, adults acquired stronger aversions than adolescents and did so at a faster rate. On a subsequent two-bottle aversion test, all morphine-injected subjects drank a significantly lower percentage of saccharin than vehicle-injected controls with adults exhibiting stronger aversions than adolescents. These age-dependent differences in morphine-induced CTAs extend the findings with other drugs of abuse for which adolescents exhibit weaker aversions. The possible basis for and implications of these differences were discussed. © 2012 Wiley Periodicals, Inc.

Keywords: adolescent; adult; morphine; taste aversion; development; deprivation

INTRODUCTION

Although the vulnerability to drug use and abuse is multifaceted, one factor which has recently received considerable attention is the age of drug exposure (Spear, 2000). Assessments of adolescent drug use are of particular importance given the host of neurobiological changes that occur during this stage of development (Spear, 2000). Considerable evidence suggests these changes may leave adolescents differentially sensitive to the affective properties of drugs and thus more likely to engage in addictive behaviors (for reviews see Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Misanin, Anderson, & Hinderliter, 2009). For example, Vastola, Douglas, Varlinskaya, and Spear (2002) demonstrated that adolescent rats exhibited a nicotine-induced place preference at .6 mg/kg nicotine, while adult rats failed to acquire a preference at this dose (for a review of place preference conditioning, see Tzschentke, 1998, 2007). Similarly, Belluzzi, Lee, Oliff, and Leslie (2004) reported that adolescents (PND38) acquired place preferences at .5 mg/kg nicotine whereas adults did not (see also Briemler, McDonald, & Smith, 2007). Additional findings with ethanol (Philpot, Badanich, & Kirstein, 2003), cocaine (Badanich, Adler, & Kirstein, 2006; Brenhouse & Andersen, 2008), and methamphetamine (Zakharova, Leoni, Kichko, & Izenwasser, 2009) mirror those of nicotine in that adolescents typically show preferences at doses lower than adults (if adults exhibit a preference at all) with the exception of amphetamine in which no apparent age differences are present (Mathews & McCormick, 2007).
Although assessments on the vulnerability to drug use and abuse generally focus on the drug’s rewarding effects, more recently it has been suggested that another affective property of drugs, that is, its aversive effect, is important in this vulnerability as well (Davis & Riley, 2010; Doremus-Fitzwater et al., 2010; Riley, 2011; Spear & Varlinskaya, 2010). Specifically, drug use is thought to be a function of the relative balance of its aversive and rewarding effects (Riley, 2011) with the drug’s aversive effects limiting overall drug intake (Stolerman & D’Mello, 1981). Conditioned taste aversion (CTA) learning is one preparation that has been used extensively to assay the aversive effects of various drugs of abuse (Freeman & Riley, 2009; for an alternative explanation, see Grigson, 1997), and interestingly such effects have been reported to differ as a function of age (see Doremus-Fitzwater et al., 2010; Misanin et al., 2009).

In one of the early reports comparing CTAs to drugs of abuse in adolescent and adult rats, Infurna and Spear (1979) reported that periadolescents (PND35) exhibited weaker amphetamine-induced aversions than young adult (PND52) rats, although the two age groups did not differ in relation to the acquisition of an amphetamine-induced place preference (Mathews & McCormick, 2007). More recently, such differential acquisition of drug-induced aversions (adolescents < adults) has been reported with cocaine (Schramm-Sapyta, Morris, & Kuhn, 2006), THC (Schramm-Sapyta et al., 2007), and ethanol (Anderson, Varlinskaya, & Spear, 2010; Schramm-Sapyta et al., 2010; Vetter-O’Hagen, Varlinskaya, & Spear, 2009). Interestingly, Shram, Funk, Li, and Lê (2006) demonstrated that adolescents exhibit weaker nicotine-induced aversions than adults (see also Wilmouth & Spear, 2004) while expressing stronger nicotine-induced place preferences (Shram et al., 2006; see also Torres, Tejeda, Natividad, & O’Dell, 2008), suggesting that adolescents are less sensitive to the aversive properties of drugs and more sensitive to their rewarding properties (see Doremus-Fitzwater et al., 2010). Although age differences are consistently seen with a variety of drugs of abuse, the findings with classical emetics such as LiCl are mixed, that is, adolescents either show weaker CTAs or no difference relative to adults (see Baker, Baker, & Kesner, 1977; Balcom, Coleman, & Norman, 1981; Guanowsky, Misanin, & Riccio, 1983; Klein, Domato, Hallstead, Stephens, & Mikulka, 1975; Klein, Mikulka, Domato, & Hallstead, 1977; Martin & Timmins, 1980; Misanin, Blatt, & Hinderliter, 1985; Misanin, Greider, & Hinderliter, 1988; Misanin, Guanowsky, & Riccio, 1983; Valliere, Misanin, & Hinderliter, 1988). Such inconsistencies are likely a function of the dose used in establishing aversions in that Schramm-Sapyta et al. (2006) reported that adolescents exhibit weaker LiCl-induced taste aversions at low-to-moderate doses, while exhibiting no differences at higher doses, suggesting that adolescents are less sensitive to the aversive effects of LiCl but can acquire comparable aversions at higher doses.

Although age-dependent differences in the acquisition of CTAs have been noted for LiCl and numerous drugs of abuse (cocaine, nicotine, amphetamine, THC, ethanol), direct comparisons of adolescents to adults in regard to opioid-induced aversions have not yet been investigated despite the abuse of such compounds by adolescent populations (Johnston, O’Malley, Bachman, & Schulenberg, 2010). In this context, morphine (like amphetamine) is interesting in that it does not appear to induce age-dependent place preferences, suggesting that it is not differentially rewarding in adolescent and adult rats (Campbell, Wood, & Spear, 2000). Given that drug use and abuse may be a function of the balance of a drug’s rewarding and aversive effects, it remains important to assess any age-dependent differences in morphine’s aversive effects that could impact drug intake. To address this possibility, the present experiments examined the ability of morphine sulfate to induce taste aversions in adolescent and adult rats under two different procedural variations. Specifically, adolescent (Experiment 1A) and adult (Experiment 1B) rats were initially restricted to 20-min daily fluid access (high deprivation) before being given a novel saccharin solution to drink and subsequently injected with various doses of morphine to assess its ability to induce age-dependent aversions. To address the possibility that restricted fluid access and the concomitant weight loss could differentially influence the ability of adolescents and adults to acquire and/or display aversions, different groups of adolescents (Experiment 2A) and adults (Experiment 2B) were examined for their ability to acquire morphine-induced aversions under a low deprivation procedure in which water was restricted to 50% of ad libitum consumption.

**EXPERIMENT 1: METHODS**

**Subjects**

Sixty-four experimentally naïve male Sprague–Dawley (Harlan Laboratories; Indianapolis, IN) rats arrived at the facility weighing approximately 40 g (PND 21, n = 32) and 90 g (PND 35, n = 32). Food and water were available ad libitum unless stated otherwise (see below). Procedures recommended by the National Research Council (1996), the Committee on Guidelines for the Care and Use of Animals in Neuroscience and Behavioral Research (2003), and the Institutional Animal Care and Use Committee at American University were followed at all times.
Apparatus

Upon arrival to the animal colony, subjects were initially handled and then group housed (four rats per bin) in polycarbonate bins (23 cm × 44 cm × 21 cm). All subjects were maintained on a 12:12 light–dark cycle (lights on at 0800 hr) and at an ambient temperature of 23°C. All conditioning and testing occurred during the light phase of the light–dark cycle. During adaptation and conditioning, animals were transferred to individual hanging wire-mesh (24.3 cm × 19 cm × 18 cm) test cages but were returned to their group-housing bins after conditioning session (see details below).

Drugs

Morphine sulfate (generously supplied by the National Institute on Drug Abuse) was dissolved in sterile isotonic saline (9%) at a concentration of 5 mg/ml and was subsequently filtered through a .2 μm filter to remove any contaminants before being administered subcutaneously (SC) at a dose of 3.2, 10, or 18 mg/kg. Sterile isotonic saline was also filtered before being administered to vehicle controls equivalent to the highest dose of morphine administered (18 mg/kg). Volume of the injection was manipulated in favor of concentration given the influence concentration has on the absorption/distribution of the drug.

**Experiment 1: Procedure**

**Experiment 1A: Adolescents.** Subjects were brought into the facility at PND 21 and were maintained on ad libitum food but were immediately water restricted. Subjects were weighed and handled on this day (PND 21), and experimental procedures began on PND 22. On this day, subjects were removed from their group-housed bins, weighed, and placed in the individual test cages. A 50-ml graduated Nalgene tube containing tap water was then affixed to each cage for 40 min. After water access, bottles were removed and consumption was recorded. Subjects remained in the test cages for an additional 20 min before being returned to their group-housed bins. This procedure was repeated for 7 consecutive days with the exception that water access was restricted to 20 min daily after the first day. On the day following adaptation, subjects were weighed and handled as previously described and given 20-min access to a novel saccharin solution (1 g/L) in the test cages after which they remained for an additional 20 min. At this point, subjects (independent of their group-housed bin) were assigned to one of four groups such that saccharin intake was comparable among groups. Based on these group assignments, subjects were injected with either morphine (3.2, 10, or 18 mg/kg SC) or vehicle (matched in volume to the high-dose group) and then returned to their group-housed bins. This procedure yielded Groups 0, 3.2, 10, and 18 for which the number indicates the dose of morphine administered. The following 2 days served as water-recovery days, and subjects were treated as described during the 7 days of water adaptation. They received no injections on these days. This 3-day cycle (conditioning/water-recovery) was repeated four times. On the day following the completion of the fourth 3-day cycle, subjects were removed from their group-housed bins and placed in their respective test cages (as previously described) and given access to two 50-ml Nalgene tubes (one containing tap water and the other containing saccharin) for 20 min. Specifically, both the saccharin- and water-filled Nalgene tubes were placed on the test cages simultaneously with the placement of the tubes (left or right side) counterbalanced across subjects to prevent positioning effects. After 20 min, the bottles were removed, consumption was recorded, and subjects were returned to their group-housed bins.

For the adolescent subjects, adaptation to the test cages and restricted fluid access took place from PND 22 to 28, saccharin pairings took place from PND 29 to 40, and the two-bottle test was given on PND 41.

**Experiment 1B: Adults.** The procedures described above were identical for the adult rats with the following exceptions. Subjects were brought into the facility at PND 35 and maintained on ad libitum food and water until PND 77 to permit the control of their developmental environment (housing conditions, handling, and light/dark cycle). Subjects were weighed and handled on PND 77 but had their bottles removed so that experimental procedures began on PND 78. Adaptation to the test cages and restricted fluid access took place from PND 78 to 84, saccharin pairings took place from PND 85 to 96, and the two-bottle test was given on PND 97. Two subjects from the high-dose group died within 24 hr after the second saccharin-drug pairing. All data from these subjects were removed from the subsequent analyses.

**Statistical Analysis**

For each age group, saccharin consumption (ml) on the four conditioning sessions was analyzed using a 4 (Dose) × 4 (Session) mixed ANOVA. To determine if there were differences in saccharin consumption (ml) between conditioning Sessions 1 and 4, Bonferroni-corrected *t*-tests were employed. A 4 (Dose) × 8 (Session) mixed ANOVA was used to analyze water consumption during recovery days. One-way ANOVAs and Tukey’s HSD post hoc tests were employed to evaluate differences in the percent saccharin consumed between the different dose groups on the two-bottle test. All statistical analyses were based on significance level of *α* = .05.

**RESULTS**

**Experiment 1A: Adolescents**

**Acquisition.** The 4 × 4 mixed ANOVA on saccharin consumption (ml) during conditioning revealed significant effects of Dose [F(3, 84) = 12.561, *p* < .05] and Session [F(3, 84) = 19.269, *p* < .05] as well as a significant Dose × Session interaction [F(9, 84) = 6.646, *p* < .05] (Fig. 1A). A subsequent one-way ANOVA indicated significant differences in consumption between the groups on Sessions 3 [F(3, 31) = 8.903, *p* < .05] and 4 [F(3, 31) = 23.741, *p* < .05]. Tukey’s post hoc
FIGURE 1 Mean (±SEM) saccharin consumption (ml) by adolescents during acquisition (A) and mean (±SEM) percent saccharin consumed on the two-bottle test (B). For acquisition, there were significant effects of Dose and Session as well as a significant Dose × Session interaction (p’s < .05). Subsequent multiple comparisons revealed that on Session 3 Groups 10 and 18 drank significantly less saccharin than Group 0. By conditioning Session 4, all morphine-treated subjects drank significantly less saccharin relative to Group 0 with Groups 10 and 18 drinking less than Group 3.2. With regard to the two-bottle test, Groups 3.2, 10, and 18 all drank a significantly lower percent of saccharin than Group 0 with Group 18 drinking a significantly lower percent relative to Group 3.2 (p’s < .05). *Significant differences between Group 0 and Groups 3.2, 10, and 18. †Significant differences between Groups 3.2 and 10. #Significant differences between Groups 0 and 10/18 (p’s < .05).

Experiment 1B: Adults

Acquisition. The 4 × 4 mixed ANOVA on saccharin consumption (ml) during conditioning revealed significant main effects of Dose [F(3, 26) = 22.919, p < .05] and Session [F(3, 78) = 42.608, p < .05] as well as a significant Dose × Session interaction [F(9, 78) = 13.344, p < .05] (Fig. 2A). The subsequent one-way ANOVA indicated significant differences in consumption between the groups on Sessions 2 [F(3, 29) = 10.104, p < .05], 3 [F(3, 29) = 27.384, p < .05], and 4 [F(3, 29) = 31.097, p < .05]. Multiple comparisons revealed that on Session 2 all morphine-injected subjects drank significantly less than Group 0 (p’s < .05). On Sessions 3 and 4, all morphine-injected subjects drank significantly less saccharin than Group 0 (p’s < .05) and Group 18 drank significantly less saccharin than Group 3.2 (p < .05). Subsequent within-subject analyses (with Bonferroni corrections) indicated that Group 0 increased consumption between Sessions 1 and 4 [t(7) = −3.610, p < .05], while Group 3.2 showed no significant change over sessions [t(7) = −3.009, p > .05]. Groups 10 and 18 significantly decreased saccharin consumption over these sessions [t(7) = 9.636, p < .05] and [t(5) = 6.089, p < .05, respectively].

There were no significant differences in water consumption among groups on the water-recovery day immediately preceding each conditioning session (all p’s > .05; data not shown).

Two-Bottle Test. A one-way ANOVA on the percent saccharin consumed on the two-bottle test revealed significant differences among groups [F(3, 31) = 16.171, p < .05] (Fig. 1B). Specifically, all morphine-injected subjects drank a significantly lower percent of saccharin than Group 0. In addition, Group 18 drank a significantly lower percent of saccharin than Group 3.2 (p’s < .05).
immediately preceding each conditioning session (all p’s > .05; data not shown).

**Two-Bottle Test.** A one-way ANOVA on the percent saccharin consumed on the two-bottle test revealed significant differences among groups \[ F(3, 29) = 38.763, p < .05 \] (Fig. 2B). Specifically, all morphine-injected subjects drank a significantly lower percent of saccharin than Group 0 (p’s < .05). On conditioning Sessions 3 and 4, all morphine-treated subjects drank significantly less saccharin relative to Group 0 with Group 18 drinking less saccharin than Group 3.2. With regard to the two-bottle test, Groups 3.2, 10, and 18 all drank a significantly lower percent of saccharin than Group 0 (p’s < .05). *Significant differences between Group 0 and Groups 3.2, 10, and 18.

**Adolescent and Adult Comparison**

An independent-samples t-test on saccharin consumption (ml) of the adolescent and adult vehicle groups on the first saccharin conditioning session revealed significant differences between adolescents and adults [t(14) = 11.092, p < .05]. To allow for a direct comparison between the adolescents and adults, consumption for the drug-injected groups was transformed to a percent of the average consumption of Group 0 across each age group for each conditioning session (Fig. 3A). Specifically, for each session, consumption in each age and dose group was calculated as a percent change from the average absolute consumption of the vehicle-injected controls (Group 0) on that session. A 2 (Age) × 3 (Dose) × 4 (Session) mixed ANOVA on these percent differences revealed significant main effects of Age [F(1, 40) = 51.498, p < .05], Dose [F(2, 40) = 6.412, p = .05], and Session [F(3, 120) = 126.982, p < .05] as well as significant interactions. There were no age differences in the percentage of saccharin consumed (relative to vehicle controls). From Sessions 2 to 4, the percent change in saccharin consumed was significantly greater for adults than adolescents, reflective of the acquisition of stronger aversions in the adult subjects.

Additionally, Bonferroni-corrected independent sample t-tests used to examine age differences in saccharin preference during the two-bottle test (Fig. 3B) revealed that adolescents consumed a significantly higher percentage of saccharin relative to adults at 10 mg/kg [t(14) = 23.925, p < .0125] and 18 mg/kg [t(14) = 9.478, p < .0125] with no differences at 0 mg/kg [t(14) = 1.105, p > .05] or 3.2 mg/kg [t(14) = 3.942, p > .0125].

**DISCUSSION: EXPERIMENT 1**

Despite the documented abuse of opioids by human adolescents (Johnston et al., 2010) and the importance of the aversive affective response of drugs in shaping drug intake (Stoelman & D’Mello, 1981), no report has assessed potential age-dependent differences in the

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**FIGURE 2** Mean (±SEM) saccharin consumption (ml) by adults during acquisition (A) and mean (±SEM) percent saccharin consumed on the two-bottle test (B). For acquisition, there were significant main effects of Dose and Session as well as a significant Dose × Session interaction (p’s < .05). Subsequent multiple comparisons revealed that on Session 2 all morphine-treated subjects drank significantly less saccharin than Group 0 (p’s < .05). On conditioning Sessions 3 and 4, all morphine-treated subjects drank significantly less saccharin relative to Group 0 with Group 18 drinking less saccharin than Group 3.2. With regard to the two-bottle test, Groups 3.2, 10, and 18 all drank a significantly lower percent of saccharin than Group 0 (p’s < .05). *Significant differences between Group 0 and Groups 3.2, 10, and 18. ^Significant differences between Groups 3.2 and 18 (p’s < .05).
ability of morphine to condition taste aversions. This was addressed in Experiment 1, in which the acquisition of morphine-induced taste aversions was examined in adolescent and adult rats. As described, adults displayed significantly stronger aversions than the adolescents and acquired these aversions at a significantly faster rate.

Although suggestive of age-dependent differences in the aversive effects of morphine (for similar differences with other drugs of abuse, see Anderson et al., 2010; Infurna & Spear, 1979; Schramm-Sapyta et al., 2006, 2007, 2010; Shram et al., 2006; Vetter-O’Hagen et al., 2009; Wilmouth & Spear, 2004), there are other possible interpretations. For example, the water-deprivation procedure used to induce fluid consumption during conditioning, that is, 20-min daily access, may have differentially affected the motivation to drink in the adolescents and adults. This possibility is supported by the fact that the restricted fluid schedule impacted body weights differently in the two age groups. Specifically, adolescent and adult vehicle-injected controls weighed 90 and 319 g, respectively, at the end of the experiments (after 20 days of restricted fluid access). This resulted in adolescent and adult subjects weighing approximately 52% and 80% of age-matched subjects maintained under free-feeding procedures (Harlan Laboratories, Indianapolis, IN). These differences in body weight suggest that the adolescent rats were impacted greater by the deprivation procedure than the adults, possibly increasing the motivation to drink. Given that deprivation is a factor in the induction and expression of taste aversions (weaker aversions under more restricted deprivation; see De la Casa & Lubow, 1995; Domjan, 1972; Peck & Ader, 1974; Revusky, Pohl, & Coombes, 1980; Sengstake & Chambers, 1979; Sengstake, Chambers, & Thrower, 1978), it is possible that these potential differences in motivation may be responsible for the behavioral differences and not any differential sensitivity to the drug’s aversive effects. To address this possibility, morphine-induced taste aversions were examined in separate groups of adolescent and adult subjects maintained under a low deprivation condition (i.e., 50% of ad libitum consumption; see Anderson et al., 2010).

EXPERIMENT 2: METHODS

Subjects

The subjects were 32 adolescent and 34 adult naïve rats of the same age, sex, and supplier as those described above in §2.1. Unless otherwise specified, they were maintained under the same conditions as described in Experiment 1.

Experiment 2: Procedure

Experiment 2A: Adolescents. Subjects were brought into the laboratory on PND 21. For the first 7 experimental days,
Experiment 2B Procedure: Adults. The procedures described above were identical for the adult rats with the following exceptions. Subjects were brought into the facility at PND 35 and maintained on ad libitum food and water until PND 77 to permit the control of their developmental environment (housing conditions, handling, and light/dark cycle). Subjects were weighed and handled on PND 77–83 at which point water adaptation and aversion conditioning began (as described above). During PND 87–95, subjects were given 45-min access to tap water in the individual test cages. From PND 97 to 103, subjects were given four saccharin-drug (or vehicle) pairings until the two-bottle test, which was administered on PND 105. Two subjects from the high-dose group died within 2 hr after the first saccharin-drug pairing. All data from these subjects were removed from the subsequent analyses. Complications associated with opioid-induced hyperphagia were observed in these two subjects. Specifically, excessive intake of woodchips and food blocks were observed which has been reported in subjects administered buprenorphine (a μ-opioid agonist) and maintained on hardwood bedding (see Clark Jr., Myers, Goelz, Thigpen, & Forsythe, 1997; Jacobson, 2000). This effect was not observed in subjects maintained on grid floors or on paper during the first 9 hr postinjection (Jablonski, Howden, & Baxter, 2001). In the present assessment, morphine-treated subjects that exhibited these complications postdrug administration were lavaged with 1 ml of tap water to clear the material from their mouths.

Statistical Analysis

For each age group, saccharin consumption (ml) on the four conditioning sessions was analyzed using a 4 (Dose) \( \times 4 \) (Session) mixed ANOVA. To determine if there were differences in saccharin consumption (ml) between conditioning Sessions 1 and 4, Bonferroni-corrected \( t \)-tests were employed. One-way ANOVAs and Tukey’s HSD post hoc tests were employed to evaluate differences in the percent saccharin consumed between the different dose groups on the two-bottle test. All statistical analyses were based on significance level of \( \alpha = 0.05 \).

RESULTS

Experiment 2A: Adolescents

Acquisition. The 4 × 4 mixed ANOVA on saccharin consumption (ml) revealed significant effects of Dose \( [F(3, 28) = 3.916, p < 0.05] \) and Session \( [F(3, 84) = 6.242, p < 0.05] \) as well as a significant Dose × Session interaction \( [F(9, 84) = 3.908, p < 0.05] \) (Fig. 4A).

The subsequent one-way ANOVA indicated significant differences in consumption between the groups on Sessions 3 \( [F(3, 31) = 7.237, p < 0.05] \) and 4 \( [F(3, 31) = 4.922, p < 0.05] \). Tukey’s post hoc analysis revealed that on Sessions 3 and 4 all morphine-injected subjects drank significantly less saccharin than Group 0 (p’s < 0.05). Subsequent within-subject analyses (with Bonferroni corrections) indicated that Groups 0, 3.2, and 10 displayed no significant changes in saccharin intake from Sessions 1 to 4 \( [t(7) = -1.839, p > 0.05, t(7) = 2.005, p > 0.05, \text{ and } t(7) = 2.089, p > 0.05] \).
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Consumption (ml) revealed significant effects of Dose <p*> and significant differences among groups (p’s < .05). Subsequent multiple comparisons revealed that on Sessions 3 and 4 all morphine-treated subjects drank significantly less saccharin than Group 0. With regard to the two-bottle test, Groups 3.2, 10, and 18 all drank a significantly lower percent of saccharin than Group 0 (p’s < .05). *Significant differences between Group 0 and Groups 3.2, 10, and 18 (p’s < .05).

**Two-Bottle Test.** A one-way ANOVA on the percent saccharin consumed on the two-bottle test revealed significant differences among groups [F(3, 31) = 13.929, p < .05] (Fig. 4B). Specifically, all morphine-injected subjects drank a significantly lower percent of saccharin than Group 0 (p’s < .05).

**Experiment 2B: Adults**

**Acquisition.** The 4 × 4 mixed ANOVA on saccharin consumption (ml) revealed significant effects of Dose [F(2, 28) = 56.512, p < .05] and Session [F(3, 84) = 20.018, p < .05] as well as a significant Dose × Session interaction [F(9, 84) = 12.617, p < .05] (Fig. 5A). The subsequent one-way ANOVA indicated significant differences in consumption between groups on Sessions 2 [F(3, 31) = 24.267, p < .05], 3 [F(3, 31) = 7.237, p < .05], and 4 [F(3, 31) = 52.688, p < .05]. Tukey’s post hoc analysis revealed that on Sessions 2–4 all morphine-injected subjects drank significantly less saccharin than Group 0 (p’s < .05). Subsequent within-subject analyses (with Bonferroni corrections) indicated that Group 0 displayed no significant changes in saccharin intake from Sessions 1 to 4 [t(7) = -3.053, p > .0125], Group 3.2 [t(7) = 7.392, p < .0125], 10 [t(7) = 7.400, p < .0125], and 18 [t(7) = 5.452, p < .0125] exhibited a significant decrease in saccharin consumption over these sessions.

**Experiment 2: Adolescent–Adult Comparisons**

Consumption for the drug-injected groups was transformed to a percent of the average consumption of Group 0 across each age group for each conditioning session (Fig. 6A). Specifically, for each session consumption in each age and dose group was calculated as a percent change from the average absolute consumption of the vehicle-injected controls (Group 0) on that session. A 2 (Age) × 3 (Dose) × 4 (Session) mixed ANOVA on these percent differences revealed significant main effects of Age [F(1, 42) = 49.389, p < .05] and Session [F(3, 126) = 109.104, p < .05], as well as a significant Session × Age [F(3, 126) = 16.522, p < .05] interaction. There was no effect of Dose [F(2, 42) = .083, p > .05] and no significant Dose × Session [F(6, 126) = .514, p > .05], Age × Dose [F(2, 42) = .633, p > .05], or Session × Age × Dose [F(6, 126) = 310, p > .05] interactions. Specifically, adults drank a significantly lower percentage of saccharin relative to adolescents. On Session 1, there were no age differences in the percentage of saccharin...
consumed (relative to vehicle controls). From Sessions 2 to 4, the percent change in saccharin consumed was significantly greater for adults than adolescents, reflective of the acquisition of stronger aversions in the adult subjects.

Additionally, Bonferroni-corrected independent sample t-tests used to examine age differences in saccharin preference during the two-bottle test (Fig. 6B) revealed that adolescents consumed a significantly higher percentage of saccharin relative to adults at 3.2 mg/kg \( [t(14) = 12.281, \ p < .0125] \) and 10 mg/kg \( [t(14) = 25.847, \ p < .0125] \) with no differences at 0 mg/kg \( [t(14) = 4.909, \ p > .0125] \) and 18 mg/kg \( [t(14) = .003, \ p > .0125] \).

**DISCUSSION: EXPERIMENT 2**

To assess the possible effects of differences in motivation on the differences in morphine-induced taste

![FIGURE 5](image_url)

**FIGURE 5** Mean (±SEM) saccharin consumption (ml) by adults during acquisition (A) and mean (±SEM) percent saccharin consumed on the two-bottle test (B). For acquisition, there were significant main effects of Dose and Session as well as a significant Dose \( \times \) Session interaction \( (p’s < .05) \). Subsequent multiple comparisons revealed that on Sessions 2–4 all morphine-treated subjects drank significantly less saccharin than Group 0 \( (p’s < .05) \). With regard to the two-bottle test, all morphine-treated subjects drank a significantly lower percent of saccharin than Group 0 with Group 18 drinking a significantly lower percent relative to Group 3.2 \( (p’s < .05) \). *Significant differences between Group 0 and Groups 3.2, 10, and 18 \( (p’s < .05) \). *Significant differences between Groups 18 and 3.2 \( (p < .05) \).

![FIGURE 6](image_url)

**FIGURE 6** Mean (±SEM) percent change in saccharin consumed (ml) from vehicle controls over repeated conditioning sessions (A) and mean (±SEM) percent saccharin consumed on the two-bottle test (B) for adolescents (white bars) and adults (black bars). There were significant main effects of Age, Session, and a significant Session \( \times \) Age interaction \( (p’s < .05) \), but no significant effects of Dose or significant Age \( \times \) Session \( \times \) Dose \( \times \) Session interactions \( (p’s > .05) \). Specifically, adults drank a significantly lower percentage of saccharin relative to adolescents. On conditioning Session 1, there were no differences in the percent change in saccharin consumed from vehicle controls, while from Sessions 2 to 4, the percent saccharin consumed by adults was significantly less than adolescents. For the two-bottle test, adults in Groups 3.2 and 10 drank a significantly lower percentage of saccharin relative to adolescents treated with the same dose \( (p’s < .05) \). *Significant differences between adolescents and adults of the same dose group \( (p’s < .05) \).
aversions between the two age groups (as reported in Experiment 1), morphine-induced taste aversions were assessed under a low deprivation procedure (Experiment 2). Specifically, adolescent and adult animals were given 50% of their ad libitum levels of fluid consumption prior to aversion conditioning with morphine (see Anderson et al., 2010). Unlike Experiment 1, the two groups displayed minimal loss of body weight under these deprivation conditions and further did not differ from each other. Adolescent and adult vehicle-injected controls weighed approximately 95% and 94% of free-feeding rats, respectively (as compared to Experiment 1 where adolescents and adults were 52% and 80% of free-feeding).

Although body weight changes in response to deprivation were comparable for adolescents and adults under the low deprivation schedule of Experiment 2, adults acquired the aversions significantly faster and exhibited stronger aversions than adolescents. That the adults continued to display significantly greater morphine-induced aversions than the adolescents under low deprivation where the two groups did not differ in their body weight changes suggests that these differences are a function of differential sensitivity to the aversive effects of morphine and not an artifact of differences in motivation.

**GENERAL DISCUSSION**

Considerable evidence suggests that the host of neurobiological changes which occur during adolescence may leave individuals in this age group differentially sensitive to the affective properties of drugs and thus more likely to engage in addictive behaviors (for reviews see Doremus-Fitzwater et al., 2010; Misanin et al., 2009). Given the abuse of opioid compounds by adolescent populations (Johnston et al., 2010) and the role the aversive properties play in shaping overall drug intake (Stolerman & D’Mello, 1981), any age differences in the aversive effects of morphine may leave adolescents more sensitive to its use and abuse. To address this issue, the present report assessed the ability of morphine to induce aversions in both adolescents and adults in high- and low-deprivation conditions. As described, both adolescents and adults acquired morphine-induced taste aversions with adolescents taking longer to acquire aversions that were weaker than adults. These age-dependent differences with morphine parallel those reported in a number of studies assessing age differences in the affective properties of other drugs of abuse (Anderson et al., 2010; Infurna & Spear, 1979; Schramm-Sapyta et al., 2006, 2007, 2010; Shram et al., 2006; Vetter-O’Hagen et al., 2009; Wilmouth & Spear, 2004).

The present data, when taken in conjunction with these previous reports, suggest some general developmental phenomenon that generalizes across a number of drugs and procedural variations, possibly a differential sensitivity to the aversive effects of such drugs. Although possible, there are several other interpretations for the reported differences in aversion learning between the two age groups. Given that the taste aversion design is one that is based in conditioning (see Garcia, McGowan, Ervin, & Koelling, 1968), any age-dependent differences in taste processing, learning, and/or retention could mediate the reported differences. Work with place preference conditioning (often with the same compounds), however, has shown that adolescents acquire such preferences more rapidly and at lower doses (Belluzzi et al., 2004; Brielmaier et al., 2007; Vastola et al., 2002; though see Campbell et al., 2000 for a report of no differences with morphine and cocaine). Any general deficits in learning and memory should be reflected in weaker effects in adolescents. Further, although taste aversions are generally weaker in adolescents than adults, when LiCl is used as the aversion-inducing compound it is often reported that there are no age-dependent differences (see Balcom et al., 1981; Guanowsky et al., 1983; Klein et al., 1977; Misanin et al., 1983; Valliere et al., 1988), revealing that adolescents are capable of processing the taste stimulus and learning and retaining the association comparably to adults (for age differences in LiCl-induced CTA see Baker et al., 1977; Klein et al., 1975; Martin & Timmins, 1980; Misanin et al., 1985, 1988).

Another possible explanation for the present data could be due to age-dependent differences in morphine’s pharmacokinetics. Interestingly, younger rats (PND 26, 32, 42) are reported to have significantly lower plasma levels of morphine relative to adults (see Johannesson & Becker, 1973; Spratto & Dorio, 1978; Wang et al., 2005). Further, Spratto and Dorio (1978) have reported that the levels of morphine in the brain are lower in adolescents relative to adults (Spratto & Dorio, 1978; though see Johannesson & Becker, 1973). These reports seem to suggest inherent developmental differences in the absorption, distribution, metabolism, and/or excretion of the drug (see also Wang et al., 2005). In addition to these pharmacokinetic differences, age-dependent differences in the pharmacodynamics of morphine have also been reported that could mediate the reported differences in aversion learning. For example, opiate receptor subtypes differentially develop in the rat (see Auguy-Valette, Cros, Gouarderes, Gout, & Pontonnier, 1978; Clendeninn, Petraitis, & Simon, 1976; Petrillo, Tavani, Verotta, Robson, & Kosterlitz,
degrees of stress (adolescent differential adaptation may have generated different conditions, handling, light/dark cycle) with adaptability at a young age (PND 35) to allow for experimental subjects, on the other hand, were brought into the facility and immediately adapted to the deprivation schedule prior to the initiation of conditioning. Adult animals that could underlie the reported differences in the age-dependent differences in aversion learning. Until such differences may have generated different degrees of stress (adolescent > adults) that impacted conditioning. Adult subjects, on the other hand, were brought into the facility at a young age (PND 35) to allow for experimental control of their developmental environment (e.g., housing conditions, handling, light/dark cycle) with adaptation not starting until PND 78. It is possible that this differential adaptation may have generated different degrees of stress (adolescent > adults) that impacted conditioning. Although possible, it is important to note that the work assessing the role of stress in aversion learning is actually quite mixed with the vast majority of studies illustrating that stress has no effect on the acquisition and/or expression of taste aversions (Anderson, Hinderliter, & Misgan, 2006; Bourne, Calton, Gustavson, & Schachtman, 1992; Bowers, Amit, & Gringras, 1996; Misgan, Kaufhold, Paul, Hinderliter, & Anderson, 2006; Revusky & Reilly, 1989). Importantly, a majority of this work is with adult animals using LiCl so it is difficult to conclude that adolescents would not be affected (or affected in a manner different from adults) with regard to aversions induced by drugs of abuse. In one assessment, which systematically examined the role of different stressors (isolation housing, restraint stress) on age-dependent drug-induced aversion learning, there were no significant effects on adolescent or adult ethanol-induced CTAs (Anderson et al., 2010), although the authors argued that water deprivation may have masked such effects. It is clear that additional assessments of the influence stress may have on the acquisition of drug-induced aversions in adolescence are merited.

The differences in saccharin consumption reported here are discussed in the context of differential sensitivity to the aversive effects of morphine; however, there are other interpretations of the suppression of consumption of drug-associated tastes. One such interpretation is anticipatory contrast (AC; Grigson, 1997). This model states that the reduction in consumption of a taste paired with a drug is not due to its aversive properties but rather the drug’s rewarding effects. That is, rats avoid drinking the drug-paired taste in anticipation of the drug’s greater rewarding properties. Although an interesting position, the actual support for this model is somewhat limited. In fact, recent work in a number of areas (see Schramm-Sapyta et al., 2006; Turenne, Miles, Parker, & Siegel, 1996) provides data contrary to this hypothesis. One specific area that has been problematic for the AC position is in aversion work with adolescents. According to the AC model, there should be a direct correlation between the ability of a drug to induce an aversion and the drug’s rewarding effects (given that it is these effects which are responsible for conditioned avoidance). Interestingly, for a number of compounds adolescents display greater place preferences (indicative of the drug’s rewarding effects) yet weaker taste aversions. Further, Verendeev & Riley, 2011) have recently demonstrated no correlation between morphine- or amphetamine-induced taste aversions and place preferences in individual adult animals trained in a concurrent CTA/CPP procedure. Thus, it is not clear to what extent (if any) AC underlies the differences reported here between adults and adolescents in morphine-induced taste aversions.

Independent of the specific mechanism underlying the age-dependent differences reported here with morphine, it is clear that adolescents display weaker morphine-induced aversions than adults, a finding consistent with several other drugs of abuse within this preparation. This effect was also maintained regardless of the level of deprivation employed during conditioning. Given that adolescence is characterized by a lack of behavioral inhibition with regard to risk-taking and novelty-seeking (Spear, 2000), this relative insensitivity to the aversive effects of drugs of abuse argues that adolescence is a period of increased vulnerability to drug use. It is important to continue to characterize the drugs for which such differences exist and to determine the factors that might impact this relative sensitivity, for example, sex, drug history, stress. An examination of the neurobiological and neurochemical mediation of this differential sensitivity to the aversive effects of such drugs may also be important in understanding any possible individual or genetic susceptibility to abuse. Examination of age differences in the aversive effects of drugs should parallel those made on drug reward. Such combined assessments may provide greater insights into vulnerability of use and abuse (see Riley, 2011).
NOTES

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