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Morphine-induced conditioned taste aversions: assessment of sexual dimorphism

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Abstract

Although sex differences in taste aversions have been reported with emetics such as lithium chloride (LiCl), little is known whether such findings generalize to other aversion-inducing drugs, including recreational compounds. One particular class of recreational compounds that induces taste aversions but that has not been examined for sex differences in its aversive properties is the opioids. To assess sex differences in the aversive properties of the opioids, Experiment 1 examined the acquisition and extinction of morphine-induced taste aversions in male and female rats. To determine whether the specific parametric conditions used in Experiment 1 would support sex differences in general, Experiment 2 examined possible sex differences in the acquisition and extinction of LiCl-induced taste aversions, a compound for which sex differences have been previously reported. During acquisition, male and female rats were given 20-min access to a novel saccharin solution and injected with either morphine (0, 10, 18 and 32 mg/kg sc; Experiment 1) or LiCl (0, 0.3, 0.6 and 1.2 mEq sc; Experiment 2) every fourth day for a total of four conditioning trials. During extinction, subjects were allowed access to saccharin but were not injected (for a total of eight trials). There were no sex differences in acquisition with either morphine or LiCl. There were also no sex differences in extinction with morphine; however, sex differences were found with LiCl, an effect consistent with prior assessments with this drug. The basis for and implications of the differences in the effects of sex on morphine- and LiCl-induced taste aversions were discussed.
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Keywords: Morphine; LiCl; Sex differences; Conditioned taste aversion

1. Introduction

The conditioned taste aversion preparation involves the pairing of a novel substance with a drug that over repeated trials typically results in the avoidance of that substance (see Riley and Tuck, 1985b). Although taste aversion learning is quite robust, its acquisition and extinction have been reported to be affected by a wide variety of parameters (e.g., food or fluid deprivation, one-bottle vs. two-bottle test, number of conditioning trials, temporal interval between CS and UCS; for reviews, see Klosterhalfen and Klosterhalfen, 1985; Riley, 1998). One parameter that has received attention in terms of its effects on aversion learning but that has not been as extensively examined is sexual dimorphism (Brot et al., 1992; Cailhol and Morméde, 2002; Chambers and Sengstake, 1976; Chambers et al., 1981; Choleris et al., 2000; Christian et al., 2001; Dacanay et al., 1984; Earley and Leonard, 1978; Ferrari and Riley, 1994; Green, 1969; Ingram and Corfman, 1981; Lucas and McMillen, 2002; Nachman, 1970; Peeters et al., 1992; Robbins, 1980; Sengstake et al., 1978; van Haaren and Hughes, 1990). Although initially demonstrated with lithium chloride (LiCl) in which males displayed a more rapid acquisition of the aversion (see Chambers and Sengstake, 1976), sex differences have subsequently been reported with a variety of other compounds, including delta-9-tetrahydrocannabinal (THC) (Chambers and Sengstake, 1976), cocaine (van Haaren and Hughes, 1990) and ethanol (Cailhol and Morméde, 2002; Lucas and McMillen, 2002). The relative sensitivity of males within this preparation is generally reported (for an exception, see Chaihol and Morméde, 2002), although the strength of the sex difference and its occurrence (e.g., during acquisition or extinction) vary across studies.

One particular group of psychoactive compounds that has been consistently reported to induce taste aversions

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(Bechara et al., 1993; Gaiardi et al., 1998; Goudie et al., 1982; Hunt et al., 1985, 1987; Hutchinson et al., 2000; Lancellotti et al., 2001; Parker, 1995; Riley et al., 1978; Schenk et al., 1987; Zito et al., 1988) but has not been assessed for sex differences in its aversive properties is the opioids. This is surprising given that sex-dependent differences with the opioids have been reported in a variety of other preparations, e.g., antinociception (Bartok and Craft, 1997; Boyer et al., 1998; Candido et al., 1992; Cicero et al., 1996; Craft and Bernal, 2001; Fernandez et al., 1999; Islam et al., 1993; Kepler et al., 1991; Krzanowska and Bodnar, 1999; Negus and Mello, 1999), locomotor activity (Boyer et al., 1998), discrimination learning (Craft et al., 1998, 1999), drug withdrawal (Craft et al., 1999; Cruz and Rodriguez-Manzo, 2000), drug tolerance (Craft et al., 1999), stress induced by handling (Fernandez et al., 1999; Romero and Bodnar, 1986), conditioned place preferences (Cicero et al., 2000) and self-administration (Alexander et al., 1978; Cicero et al., 2003; Hadaway et al., 1979; Hill, 1978; Klein et al., 1997; Lynch and Carroll, 1999; for alternative findings, see Stewart et al., 1996; for a review of sex differences in drug self-administration, see Lynch et al., 2002).

To address the question of sex differences in the aversive effects of the opioids, Experiment 1 examined the acquisition and extinction of morphine-induced taste aversions in fluid-deprived male and female Sprague–Dawley rats. To assess the general ability of the specific parametric conditions used in Experiment 1 to support sex differences, Experiment 2 examined the acquisition and extinction of LiCl-induced aversions in male and female rats. As noted above, sex differences with LiCl have been reported under a variety of parametric conditions (see Chambers and Sengstake, 1976; Chambers et al., 1981; Dacanay et al., 1984; Earley and Leonard, 1978; Ingram and Corfman, 1981; Robbins, 1980; Sengstake et al., 1978).

2. General method

2.1. Subjects

Subjects were 64 experimentally naïve male and 66 experimentally naïve female rats of Sprague–Dawley descent (Harlan Sprague–Dawley), approximately 120 days of age and weighing between 303–426 and 189–283 g, respectively, at the start of the experiments. Guidelines established by the Institutional Animal Care and Use Committee at American University were followed at all times.

2.2. Apparatus

Subjects were housed individually in stainless steel, wire mesh cages on the front wall of which a single 50-ml graduated Nalgene tube was placed for presentation of either water or saccharin. Subjects were maintained on a 12:12-h light/dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C for the duration of the experiments. Rat chow (Harlan Tech Laboratory) was available ad libitum.

2.3. Drugs and solutions

Morphine sulfate (generously provided by the National Institute on Drug Abuse) was prepared as a 10 mg/ml solution in distilled water. LiCl (0.15 M) (Sigma Pharmaceutical) was prepared as a 6.4 mg/ml solution in distilled water. Saccharin (0.1% sodium saccharin, Sigma) was prepared as a 1 g/l solution in tap water. All morphine, LiCl and vehicle injections were administered subcutaneously.

2.4. Procedure

2.4.1. Phase I: Habituation

Following $23^{2/3}$ h of water deprivation, all subjects were given 20-min access to water daily for 15 consecutive days.

2.4.2. Phase II: Acquisition

On Day 1 of this phase, all subjects were given access to a novel saccharin solution during the scheduled 20-min fluid-access period. Immediately following this exposure, all male subjects in each experiment were ranked on saccharin consumption and assigned to four groups, such that mean saccharin consumption was comparable among groups. Immediately following consumption, they were injected with various doses of an aversion-inducing agent (see below) or drug vehicle. Female subjects in each experiment were similarly ranked, assigned to four groups and injected with drug or vehicle. On each of the 3 days following this trial, all subjects were given 20-min access to water. This sequence of alternating a single acquisition trial with three water recovery days continued for four complete cycles. On the day following the third water recovery session of the fourth cycle, all subjects were given 20-min access to saccharin in a final aversion test. No injections were given following this test. All subjects were then given three water recovery sessions prior to the initiation of extinction (see below).

2.4.3. Phase III: Extinction

On Day 1 of this phase, all male subjects in each experiment were given access to a novel saccharin solution during the scheduled 20-min fluid-access period. No injections were given following this exposure. All female subjects were treated similarly. On each of the 3 days following this extinction trial, all subjects were given 20-min access to water. This sequence of alternating a single extinction trial with three water recovery days continued for seven complete cycles.

2.5. Experiment 1: Morphine

2.5.1. Specific procedure

During conditioning in this experiment, 23 male subjects were given access to saccharin followed by an injection of either 10, 18 or 32 mg/kg of morphine sulfate, yielding Groups M10 ($n=8$), M18 ($n=8$) and M32 ($n=7$). In addition, 24 female subjects were given access to saccharin followed by an injection of either 10, 18 or 32 mg/kg of morphine sulfate, yielding Groups F10 ($n=8$), F18 ($n=8$) and F32 ($n=8$). Groups MV ($n=8$) and FV ($n=8$), male and female subjects, respectively, were given distilled water equivolume to that given for the highest dose of drug (32 mg/kg). This specific dose range is based on prior work assessing morphine-induced taste aversions in Sprague–Dawley rats (see Bardo and Valone, 1994; Bevins and Bardo, 1998; Miller et al., 1990; Mucha and Herz, 1986). Such doses generally produce intermediate to strong non-dose-dependent suppression.

2.6. Experiment 2: LiCl

2.6.1. Specific procedure

During conditioning in this experiment, 25 male subjects were given access to saccharin followed by an injection of either 0.3, 0.6 or 1.2 mEq of LiCl, yielding Groups M0.3 ($n=8$), M0.6 ($n=8$) and M1.2 ($n=9$). In addition, 26 female subjects were given access to saccharin followed by an injection of either 0.3, 0.6 or 1.2 mEq of LiCl, yielding Groups F0.3 ($n=8$), F0.6 ($n=9$) and F1.2 ($n=9$). Groups MV ($n=8$) and FV ($n=8$), male and female subjects, respectively, were given distilled water equivolume to that given for the highest dose of drug (1.2 mEq). This specific dose range is based on prior work assessing LiCl-induced taste aversions (see Dacanay et al., 1984). Such doses generally produce graded dose-dependent suppression.

3. Results

Table 1 presents the mean absolute saccharin consumption (± SEM) for subjects in all groups during the acquisition and extinction of morphine-induced (Experiment 1) and LiCl-induced (Experiment 2) taste aversions. For each experiment, a repeated measures analysis of variance (ANOVA) with the between-subjects variables of sex (female and male), dose (Experiment 1: morphine 0, 10, 18 and 32 mg/kg; Experiment 2: LiCl 0, 0.3, 0.6 and 1.2 mEq) and trial (acquisition: Trials 1–4 and test trial; extinction: test trial and Trials 1–7) revealed that males consumed significantly more saccharin than females on the initial exposure to saccharin prior to being injected with either morphine (ts>5.90, $df=62$; $P<.05$) or LiCl (ts>3.31, $df=65$; $P<.05$). Given these initial baseline differences in saccharin consumption within each treatment condition, it is difficult to isolate the effects of sex on aversion learning (which is reflected in changes in consumption). To assess any differences between sexes within each drug condition, all consumption data for individual groups were recalculated and expressed as a percent shift from the control baseline for that condition (see Dacanay et al., 1984). That is, on each trial, saccharin consumption for each drug-treated subject was compared to the mean saccharin consumption of controls (i.e., drug-exposed subject's consump-

Table 1
Presentation of the mean (± S.E.M.) saccharin consumption (ml) for female (F) and male (M) subjects during the acquisition (Trials 1–4 and test) and extinction (Test Trials 1–7) of aversions induced by morphine (top) and LiCl (bottom)

		Acquisition					Extinction						
		Trial 1	Trial 2	Trial 3	Trial 4	Test	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Morphine													
0 mg/kg	M	15.6 ± 2.2	18.1 ± 1.6	19.8 ± 0.9	21.3 ± 1.2	21.1 ± 1.5	19.5 ± 1.1	15.5 ± 2.4	17.8 ± 2.1	21.6 ± 1.2	20.0 ± 1.9	21.3 ± 2.4	22.0 ± 1.0
	F	9.1 ± 1.2	15.0 ± 0.7	14.4 ± 0.7	13.7 ± 0.7	13.5 ± 1.0	13.6 ± 0.6	11.6 ± 1.2	10.7 ± 0.9	14.0 ± 1.2	13.3 ± 0.7	15.3 ± 0.7	15.2 ± 0.6
10 mg/kg	M	14.6 ± 1.5	7.6 ± 1.7	4.8 ± 1.8	4.0 ± 1.8	4.6 ± 2.3	4.5 ± 2.0	4.1 ± 1.9	4.9 ± 2.3	6.3 ± 2.4	8.0 ± 2.8	10.0 ± 3.1	12.6 ± 2.9
	F	9.0 ± 1.8	6.0 ± 0.9	3.0 ± 0.7	3.6 ± 1.0	3.3 ± 1.1	4.1 ± 1.0	2.4 ± 1.0	3.2 ± 1.1	6.7 ± 1.9	5.5 ± 1.7	8.2 ± 1.9	9.0 ± 1.3
18 mg/kg	M	14.4 ± 1.4	9.2 ± 1.5	4.3 ± 0.7	3.0 ± 0.8	2.7 ± 0.8	3.7 ± 1.3	1.8 ± 1.1	2.4 ± 1.2	7.4 ± 2.5	6.7 ± 2.6	11.0 ± 2.9	13.3 ± 2.9
	F	9.1 ± 1.0	6.2 ± 0.8	4.0 ± 0.9	3.3 ± 1.1	2.2 ± 1.1	4.8 ± 1.7	2.7 ± 1.4	3.7 ± 1.8	6.9 ± 1.5	6.6 ± 2.0	10.1 ± 2.4	10.1 ± 2.3
32 mg/kg	M	15.0 ± 1.3	11.1 ± 1.6	6.7 ± 1.4	1.6 ± 1.2	4.2 ± 2.0	6.3 ± 2.8	3.0 ± 1.4	5.0 ± 2.4	8.7 ± 3.5	7.7 ± 3.6	10.2 ± 4.0	12.9 ± 4.7
	F	9.1 ± 1.3	7.5 ± 1.6	3.6 ± 1.4	4.4 ± 1.2	1.2 ± 2.1	1.8 ± 2.8	0.7 ± 0.3	0.9 ± 0.6	3.6 ± 1.4	3.8 ± 1.8	6.5 ± 1.6	8.3 ± 1.5
LiCl													
0 mg/kg	M	11.0 ± 1.5	13.9 ± 1.5	14.5 ± 1.3	14.6 ± 0.9	13.1 ± 0.9	18.2 ± 1.5	19.4 ± 0.8	19.0 ± 0.8	20.4 ± 0.9	21.0 ± 1.1	22.1 ± 0.9	21.3 ± 1.1
	F	8.4 ± 1.1	11.7 ± 0.6	12.4 ± 1.0	12.1 ± 0.7	12.6 ± 0.8	14.1 ± 0.5	12.5 ± 0.5	13.9 ± 0.9	14.4 ± 1.1	14.7 ± 0.8	15.3 ± 0.8	15.0 ± 0.7
0.3 mEq	M	11.2 ± 1.3	10.8 ± 1.1	8.4 ± 1.0	7.5 ± 1.0	5.0 ± 1.9	9.6 ± 2.4	11.5 ± 2.3	14.3 ± 2.4	15.1 ± 2.6	18.0 ± 2.6	19.1 ± 2.1	19.9 ± 2.1
	F	8.5 ± 1.0	7.7 ± 0.9	7.4 ± 0.7	6.1 ± 0.7	5.5 ± 1.1	10.6 ± 1.0	11.1 ± 0.9	12.5 ± 1.0	14.3 ± 1.0	15.1 ± 0.7	16.7 ± 1.1	16.7 ± 1.1
0.6 mEq	M	11.1 ± 1.2	3.2 ± 0.9	1.1 ± 0.4	1.3 ± 0.2	0.4 ± 0.3	0.1 ± 0.1	0.9 ± 0.3	0.3 ± 0.3	0.6 ± 0.5	1.5 ± 1.1	2.3 ± 1.9	3.5 ± 2.3
	F	8.6 ± 0.9	4.6 ± 0.7	2.3 ± 0.7	1.3 ± 0.4	0.2 ± 0.1	1.7 ± 0.6	1.6 ± 1.0	2.8 ± 1.2	4.6 ± 1.8	5.8 ± 1.7	8.0 ± 2.0	9.8 ± 2.3
1.2 mEq	M	12.1 ± 1.4	3.0 ± 1.1	0.4 ± 0.3	0.3 ± 0.1	0.5 ± 0.3	0.1 ± 0.7	0.6 ± 0.5	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
	F	9.0 ± 1.0	1.5 ± 0.3	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.4 ± 0.2	1.2 ± 0.6

Specifically, during acquisition, subjects were presented with saccharin and injected with either morphine (0, 10, 18 or 32 mg/kg) or LiCl (0, 0.3, 0.6 and 1.2 mEq), and during extinction, subjects were presented with saccharin alone.

tion – control group's average consumption/control group's average consumption × 100), and a mean percent difference from controls was calculated. Differences in mean percent shift from controls among drug-treated groups during acquisition were then assessed using a 2 × 3 × 5 repeated measures ANOVA, with the between-subjects variables of sex (female and male) and dose (for Experiment 1: morphine, doses 10, 18 and 32 mg/kg; for Experiment 2: LiCl, doses 0.3, 0.6 and 1.2 mEq) and the within-subjects variable of trial (Trials 1–4 and test trial). Differences in mean percent shift from controls among drug-treated groups during extinction were similarly determined and subsequently assessed using a 2 × 3 × 8 repeated measures ANOVA, with the between-subjects variables of sex (female and male) and dose (for Experiment 1: morphine, doses 10, 18 and 32 mg/kg; for Experiment 2: LiCl, doses 0.3, 0.6 and 1.2 mEq) and the within-subjects variable of trial (Test Trial and Trials 1–7). An alpha of .05 was used for determining significance.

3.1. Experiment 1: Morphine

3.1.1. Acquisition

Fig. 1 illustrates the mean (± S.E.M.) percent shift in saccharin consumption from controls for females and males at each dose of morphine (10 mg/kg, top; 18 mg/kg, center; and 32 mg/kg, bottom) over repeated acquisition trials and on the final aversion test (test trial). Repeated measures ANOVA revealed a significant effect of trial [$F(4,164) = 259.15$, $P < .0001$]. Specifically, on conditioning Trial 2 (the first aversion assessment) and over subsequent acquisition, all drug-exposed groups decreased consumption relative to their respective control groups. There was no significant effect of sex [$F(1,41) = 0.004$, $P > .96$] or dose [$F(2,41) = 0.07$, $P > .93$]. Further, repeated measures ANOVA revealed no significant Sex × Dose [$F(2,41) = 0.33$, $P > .72$], Sex × Trial [$F(4,164) = 1.66$, $P > .16$], Dose × Trial [$F(8,164) = 1.78$, $P > .08$] or Sex × Dose × Trial [$F(8,164) = 0.41$, $P > .91$] interaction.

3.1.2. Extinction

Fig. 2 illustrates the mean (± S.E.M.) percent shift in saccharin consumption from controls for females and males at each dose of morphine (10 mg/kg, top; 18 mg/kg, center; and 32 mg/kg, bottom) on the final aversion test (test trial) and over repeated extinction trials. Repeated measures ANOVA revealed a significant effect of trial [$F(7,287) = 63.44$, $P < .0001$], with all groups increasing saccharin consumption relative to their controls over extinction. There was no significant effect of sex [$F(1,41) = 0.03$, $P > .87$] or dose [$F(2,41) = 0.22$, $P > .80$]. Further, repeated measures ANOVA revealed no significant Sex × Dose [$F(2,41) = 0.63$, $P > .54$], Sex × Trial [$F(7,287) = 0.43$, $P > .88$], Dose × Trial [$F(14,287) = 0.63$, $P > .84$] or Sex × Dose × Trial [$F(14,287) = 0.53$, $P > .91$] interaction.

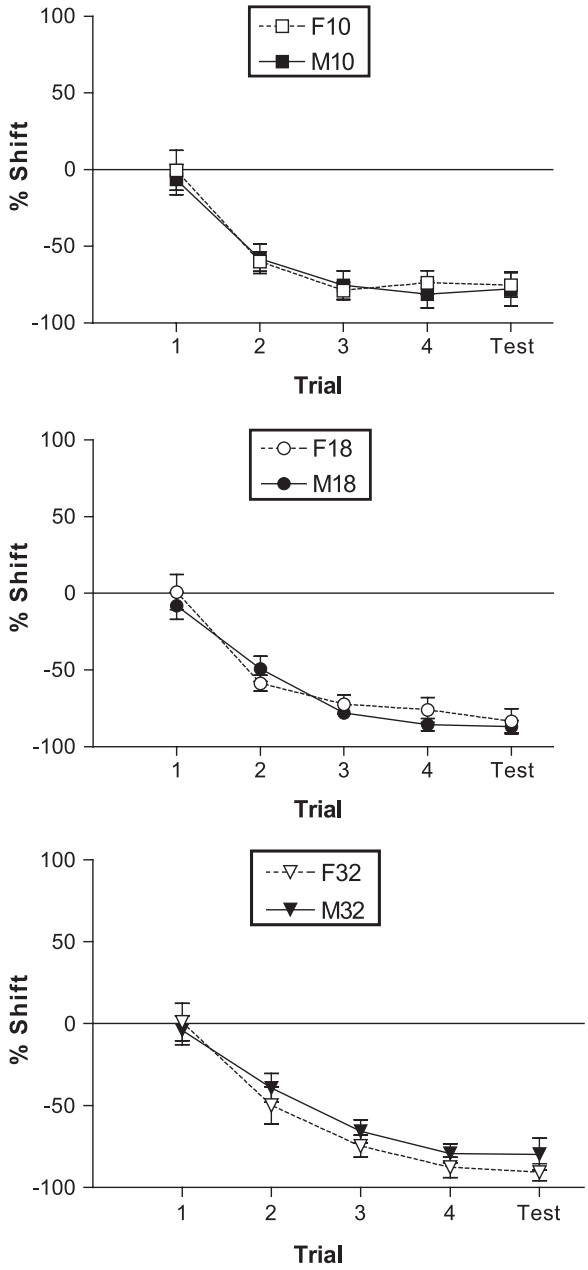

Fig. 1. Illustration of mean (± S.E.M.) percent shift from controls in saccharin consumption by female and male subjects receiving repeated pairings of saccharin with injections of morphine (10 mg/kg, top; 18 mg/kg, center; and 32 mg/kg, bottom) over acquisition. * $P < .05$.

3.2. Experiment 2: LiCl

3.2.1. Acquisition

Fig. 3 illustrates the mean (± S.E.M.) percent shift in saccharin consumption from controls for females and males at each dose of LiCl (0.3 mEq, top; 0.6 mEq, center; and 1.2 mEq, bottom) over repeated acquisition trials and on the final aversion test (test trial). Repeated measures ANOVA revealed a significant effect of trial [$F(4,180) = 202.06$, $P < .0001$] and dose [$F(2,45) = 0.40$, $P < .0001$], as well as a significant Dose × Trial [$F(8,180) = 9.35$, $P > .0001$] inter-

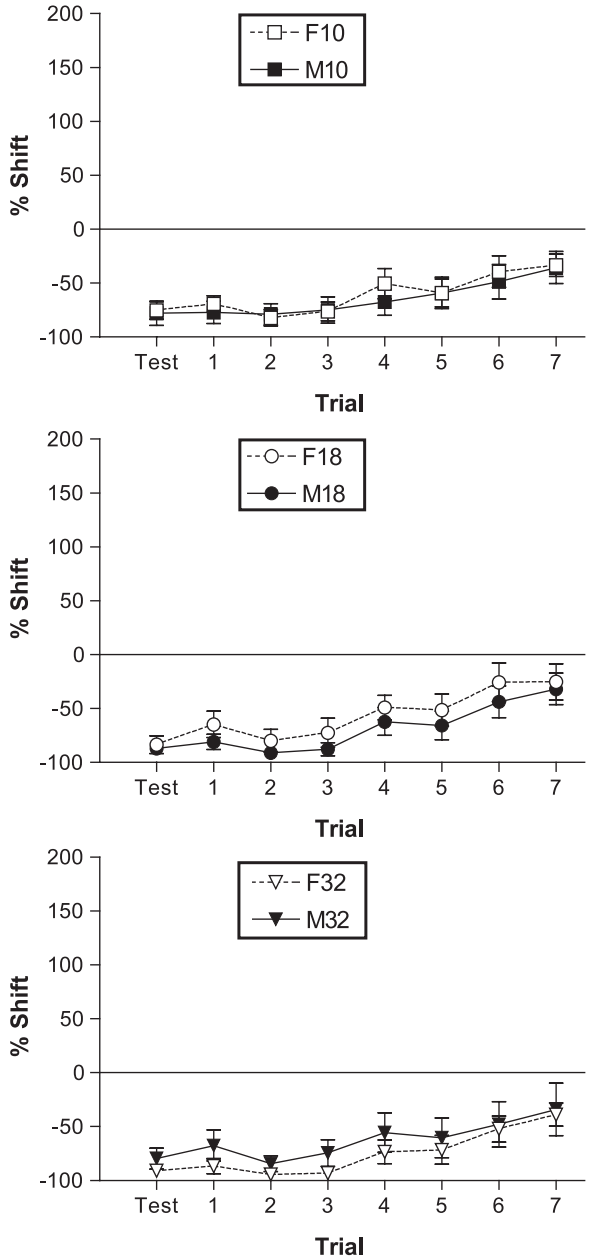

Fig. 2. Illustration of mean (± S.E.M.) percent shift from controls in saccharin consumption during extinction by female and male subjects that had previously received repeated pairings of saccharin with injections of morphine (10 mg/kg, top; 18 mg/kg, center; and 32 mg/kg, bottom) over acquisition. * $P < .05$.

action. On conditioning Trial 2 (the first aversion assessment) and over subsequent acquisition, all drug-exposed groups decreased consumption relative to their respective control groups with the strength of the aversion dependent upon the dose of LiCl. There was no significant effect of sex [$F(1,45) = 0.08$, $P > .78$]. Further, repeated measures ANOVA revealed no significant Sex × Dose [$F(2,45) = 0.99$, $P > .38$], Sex × Trial [$F(4,180) = 0.03$, $P > .89$] or Sex × Dose × Trial [$F(8,180) = 0.39$, $P > .92$] interaction.

3.2.2. Extinction

Fig. 4 illustrates the mean (± S.E.M.) percent shift in saccharin consumption from controls for females and males at each dose of LiCl (0.3 mEq, top; 0.6 mEq, center; and 1.2 mEq, bottom) on the final aversion test (test trial) and over repeated extinction trials. Repeated measures ANOVA revealed a significant effect of trial [$F(7,315) = 33.58$, $P = .0001$], sex [$F(1,45) = 8.60$, $P > .0001$] and Dose [$F(2,45) = 2.07$, $P > .0001$]. Repeated measures ANOVA also revealed a significant Sex × Trial [$F(7,315) = 4.20$,

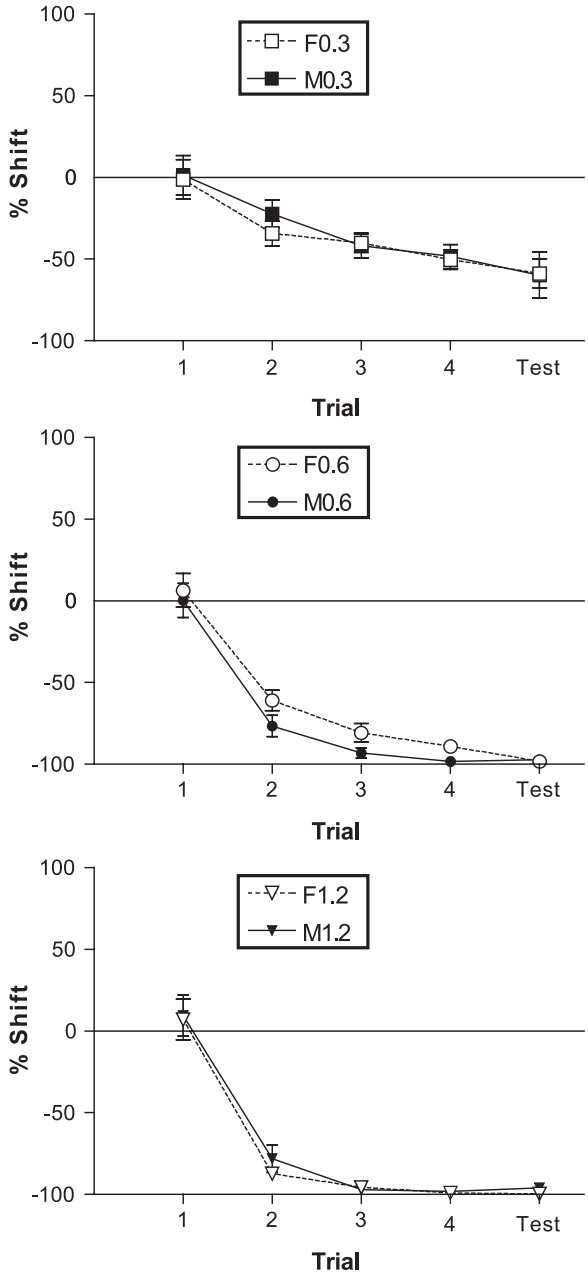

Fig. 3. Illustration of mean (± S.E.M.) percent shift from controls in saccharin consumption by female and male subjects receiving repeated pairings of saccharin with injections of LiCl (0.3 mEq, top; 0.6 mEq, center; and 1.2 mEq, bottom) over acquisition. * $P < .05$.

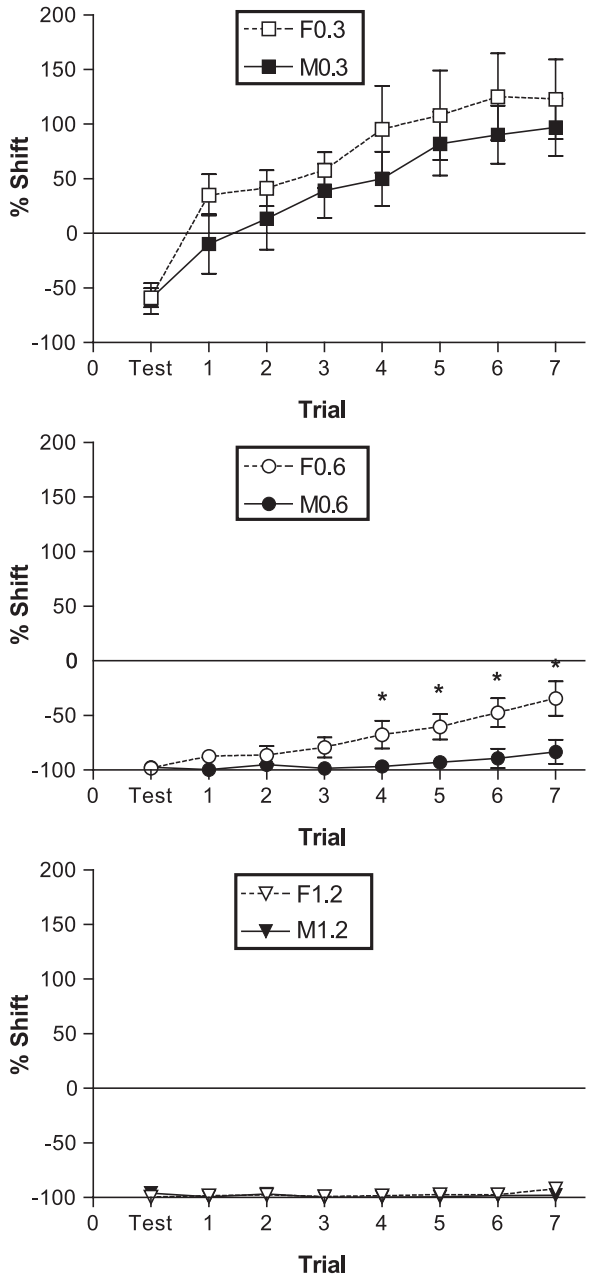

Fig. 4. Illustration of mean (± S.E.M.) percent shift from controls in saccharin consumption during extinction by female and male subjects that had previously received repeated pairings of saccharin with injections of LiCl (0.3 mEq, top; 0.6 mEq, center; and 1.2 mEq, bottom) over acquisition. * $P < .05$.

$P > .0001$], Dose × Trial [$F(14,315) = 10.31$, $P > .001$] and Sex × Dose × Trial [$F(14,314) = 2.11$, $P > .01$], but no significant Sex × Dose [$F(2,45) = 2.07$, $P > .14$], interaction. Post hoc assessments using t test comparisons revealed that on the final aversion test, there were no significant differences in the percent shift from controls between males and females at any dose of LiCl (all Ps > .98). Over subsequent trials, between-group comparisons of percent shift revealed that Groups F0.3 and M0.3 (ts < 2.16, $df = 15$; Ps > .35) and Groups F1.2 and M1.2 (ts < 1.90, $df = 15$; Ps > .08) did not differ. Differences were found, however, between Groups F0.6 and M0.6. Specifically, on Trials 4–7, the percent shift from controls was significantly less for females given 0.6 mEq LiCl than males given 0.6 mEq LiCl (ts > 8.11, $df = 15$; Ps < .05), indicative of a faster extinction for females. These differences in the percent shift from controls between males and females at this dose do not reflect relative differences in body weight for these groups from control animals. Within each sex, there was no relationship between relative body weight (from controls) or absolute body weight and the amount consumed during acquisition or extinction (data not shown).

4. Discussion

In Experiment 1, both male and female subjects given repeated pairings of saccharin and morphine acquired an aversion to the morphine-associated taste. Consistent with other work on morphine-induced taste aversions (see Bechara et al., 1993; Gaiardi et al., 1998; Goudie et al., 1982; Hunt et al., 1985, 1987; Hutchinson et al., 2000; Lancellotti et al., 2001; Parker, 1995; Riley et al., 1978; Schenk et al., 1987; Zito et al., 1988), these aversions strengthened over trials (though see Siegel et al., 1995). Also consistent with prior work with morphine in the taste aversion preparation, there was minimal effect of dose on the degree of aversions acquired (for related findings, see Bardo and Valone, 1994; Lancellotti et al., 2001; Riley et al., 1978; Siegel et al., 1995). Further, the acquisition of these aversions did not appear to be dependent upon the sex of the subject as males and females acquired the aversions at comparable rates (when comparing differences in the percent shift from controls). When saccharin was no longer paired with morphine, both male and female subjects extinguished their aversion to saccharin, eventually drinking at control levels. Further, this extinction occurred at comparable rates for males and females (again, when comparing differences in the percent shift from controls) with no effect of conditioning dose.

The failure to see sex differences in morphine-induced conditioned taste aversions during acquisition and extinction may have been the result of the specific parametric conditions under which the assessments were made, e.g., deprived subjects receiving limited fluid access in a one-bottle procedure. As previously discussed, conditioned taste aversion learning is a function of a variety of parametric conditions (for reviews, see Klosterhalfen and Klosterhalfen, 1985; Riley, 1998), and it is possible that the specific combination of parameters used in the present experiment precluded seeing clear sex-dependent differences. To address this issue, Experiment 2 examined under the same parametric conditions as those used in the assessment with morphine, the acquisition and extinction of aversions induced by LiCl, a compound for which sex differences have previously been reported (see Chambers and Sengstake,

1976; Chambers et al., 1981; Dacanay et al., 1984; Earley and Leonard, 1978; Ingram and Corfman, 1981; Robbins, 1980; Sengstake et al., 1978). As described, both male and female subjects given repeated pairings of saccharin and LiCl acquired an aversion to the LiCl-associated taste. Consistent with other work on LiCl-induced taste aversions, these aversions strengthened over trials (Dacanay and Riley, 1982; Kulkosky et al., 1980) and were dose dependent (see Dacanay et al., 1984). Similar to the effects reported with morphine (see above), there were no sex differences in the acquisition of LiCl-induced aversions (when comparing differences in the percent shift from controls). Sex differences, however, were found during the extinction of LiCl-induced aversions. Specifically, at 0.6 mEq LiCl, females drank significantly more than males in comparison to their respective control groups over Trials 4–7 (see Chambers and Sengstake, 1976; Chambers et al., 1981; Sengstake et al., 1978), i.e., females extinguished the LiCl aversion faster than males. These differences are similar to those reported by others who have found differences between males and females with LiCl. Interestingly, such differences are generally found only at specific doses, e.g., 0.3 mEq LiCl (see Chambers and Sengstake et al., 1976; Chambers et al., 1981; Dacanay et al., 1984; Robbins, 1980; Sengstake et al., 1978) and only during extinction (Chambers and Sengstake, 1976; Chambers et al., 1981; Robbins, 1980; Sengstake et al., 1978; though see Dacanay et al., 1984).

Although the fact that sex differences are seen with LiCl, but not morphine, might be interpreted that such effects are due to the specific drug examined (i.e., drug dependency), it is important to note that aversion learning in general, and sex differences more specifically, are a function of a number of parametric conditions (see Klosterhalfen and Klosterhalfen, 1985; Riley, 1998). For example, when LiCl has been used as the aversion-inducing agent, sex differences are not always reported (Chambers and Sengstake, 1976; Earley and Leonard, 1978; Green, 1969; Lucas and McMillen, 2002; Nachman, 1970). Studies assessing LiCl differ on a range of parameters, e.g., dose of drug, route of administration, number of trials and degree of deprivation (see Chambers and Sengstake, 1976; Chambers et al., 1981; Earley and Leonard, 1978; Ingram and Corfman, 1981; Robbins, 1980; Sengstake et al., 1978), indicating that such conditions may affect the likelihood of seeing sex differences. One parametric variation that has been shown to affect sex differences with LiCl is dose (see above; see also Experiment 2). Given the importance of dose in sex differences with LiCl, it is possible that had other doses of morphine been given, sex differences would have been seen with this compound as well. Interestingly, lower doses of morphine than those used in the present experiment (i.e., less than 8–10 mg/kg) generally are ineffective in inducing aversions (see Bardo and Valone, 1994; Bevins and Bardo, 1998; Siegel et al., 1995; though see Mucha and Herz, 1985; Revusky and Reilly, 1989) or induce aversions in a manner not thought to be mediated by opioid receptor activity (Mucha and Herz, 1986). Higher doses than those used in the present assessment (greater than 32 mg/kg) generally produce aversions comparable to those reported at intermediate doses (see Siegel et al., 1995; see Riley et al., 1978 for similar findings in Long–Evans rats). Although suggestive that an examination of a broader dose range would not reveal additional sex differences, until such an examination is made it may be premature to conclude that there are no sex differences with morphine or that the differences between morphine and LiCl in relation to sex effects are due to the nature of the drug. Further, it remains unknown to what extent other factors that may be equated across drug assessments, e.g., isolation housing, deprivation schedule, testing conditions, estrous cycle, might differentially interact with a specific drug to affect aversion learning. Such differential effects would argue against a simple position of drug-dependent sex differences in aversion learning.

Although sex differences were not found in morphine's aversive effects, such differences have been reported in the rewarding effects of morphine and other opioids (Alexander et al., 1978; Cicero et al., 2000, 2003; Hadaway et al., 1979; Hill, 1978; Klein et al., 1997; Lynch and Carroll, 1999; though, see Stewart et al., 1996; for a review of sex differences in drug self-administration, see Lynch et al., 2002). For example, Cicero et al. (2000) showed that although both male and female Sprague–Dawley rats were able to acquire morphine-induced place preferences, females displayed an increase while males displayed a decrease in preferences over increasing doses of morphine. In a related and more recent examination of sex differences in morphine and heroin self-administration, Cicero et al. (2003) found that female Sprague–Dawley rats acquired heroin self-administration in fewer trials than male Sprague–Dawley rats and that females displayed significantly higher breakpoints than males for both morphine and heroin (see also Alexander et al., 1978; Hadaway et al., 1979; Hill, 1978; Klein et al., 1997; Lynch and Carroll, 1999; though see Stewart et al., 1996). Given that drug acceptability is thought to be a function of the balance between a drug's rewarding and aversive effects (see Goudie et al., 1978; Grakalic and Riley, 2002; Hunt and Amit, 1987), the present data suggest that the reported differences in opioid self-administration between males and females might best be considered a function of differences in the drug's rewarding effects. Although suggestive, such a conclusion must be cautiously made. Specifically, the dose, route and sequencing of injections given in the present experiment to assess sex differences in the aversive effects of morphine are quite different from those used in the assessment of morphine reward or in the assessment of opioid self-administration. Until sex differences in the aversive effects of morphine are assessed under conditions in which morphine is self-administered, it remains unknown to what extent (if any) the aversive effects of morphine modulate opioid self-administration and if these effects vary with sex. Further, it remains to be determined if (and to what degree) reported sex

differences in aversions induced by other compounds impact sex differences in the self-administration of those drugs.

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