A history of morphine-induced taste aversion learning fails to affect morphine-induced place preference conditioning in rats

Heather E. King · Anthony L. Riley

Abstract Drugs of abuse have both rewarding and aversive effects, as indexed by the fact that they support place preferences and taste aversions, respectively. In the present study, we explored whether having a history with the aversive effects of morphine (via taste aversion conditioning) impacted the subsequent rewarding effects of morphine, as measured in the place preference design. In Experiment 1, rats were exposed to a taste aversion procedure in which saccharin was followed by morphine. Place preference conditioning was then initiated in which animals were injected with morphine and placed on one side of a two-chambered apparatus. Animals with a taste aversion history acquired place preferences to the same degree as controls without such a history, suggesting that morphine's affective properties condition multiple effects, dependent on the specific stimuli present during conditioning. To determine whether these results were a reflection of processes operating in traditional associative conditioning, in a modified blocking procedure, place preference conditioning was attempted in the presence of a taste previously associated with morphine (Exp. 2). Under these conditions, animals still acquired morphine-induced place preferences comparable to those of animals without a morphine or conditioning history. These results are consistent with the position that drugs of abuse have multiple stimulus effects (positive and negative) that are differentially associated with specific stimuli (environmental and taste) that drive different behavioral responses (approach and avoidance).

Keywords Taste aversion learning · Place preference conditioning · History · Morphine

H. E. King (✉) · A. L. Riley
Department of Psychology, Psychopharmacology Laboratory,
American University, 4400 Massachusetts Ave NW, Washington,
DC 20016, USA
e-mail: hk9905a@american.edu

In 1955, Garcia and his colleagues reported that animals given access to a novel saccharin solution and then subjected to low-dose ionizing radiation avoided consumption of saccharin on subsequent exposures (see Garcia, Kimeldorf, & Koelling, 1955). This avoidance presumably reflected the association of the taste of saccharin with the aversive effects of the radiation—that is, a conditioned taste aversion (for a review of the history of taste aversion learning, see Freeman & Riley, 2009; for an alternative interpretation, see Grigson, 1997). Although initially reported with X-irradiation, such learning has since been demonstrated with a variety of agents. For example, Garcia and Koelling (1967) examined the ability of a variety of classical emetics such as apomorphine and lithium chloride (LiCl) to condition aversions to a novel saccharin solution. Under both conditions, aversions were established, leading Garcia and his colleagues to conclude that gastrointestinal illness (whether induced by radiation or classical emetics) was sufficient to induce taste aversions (see Garcia & Ervin, 1968).

Subsequently, such aversions have been demonstrated with drugs that were reported to be self-administered (and thus classified as rewarding)—for example, alcohol (Lester, Nachman, & Le Magnen, 1970), amphetamine and mescaline (Cappell & LeBlanc, 1971), Δ9-tetrahydrocannabinol (Elsmore & Fletcher, 1972), morphine (Cappell, LeBlanc, & Endrenyi, 1973), methamphetamine (Martin & Ellinwood, 1973), and cocaine (Cappell & Le Blanc, 1975). The fact that drugs of abuse could also support taste aversion conditioning was at first considered to be paradoxical (Goudie, 1979; see also Hunt & Amit, 1987). Others suggested that these apparently paradoxical effects might simply reflect the different parametric conditions (e.g., dose of drug, route of administration, drug duration) under which the rewarding and aversive effects were assessed. In direct tests of the latter position, however, it was reported that the rewarding and aversive effects of such compounds could be demonstrated in the same animal at the same dose, route of administration, and

duration, and were not simply a function of parametric differences between the two assessments (see White, Sklar, & Amit, 1977; Wise, Yokel, & DeWitt, 1976). That rats avoid tastes associated with drugs of abuse yet prefer environments in which the drug is given suggests that such drugs have multiple stimulus effects—that is, aversive and rewarding—with the conditioned response being a function of the specific stimuli with which the drug is paired (Bechara & Van der Kooy, 1985; Garcia & Koelling, 1966; Garcia, McGowan, Ervin, & Koelling, 1968).

Although drugs of abuse have both aversive and rewarding effects, little is known about the extent (if any) to which experience with one effect might impact the other. If these affective properties both occur and are selectively associated with specific stimuli (e.g., taste and place, respectively), it might be expected that prior experience with morphine-induced taste aversions would have no effect on the ability of morphine to induce a place preference. Interestingly, in a different associative preparation, Escobar, Matute, and Miller (2001) reported that animals given serial associations involving the same outcome displayed interference (both retroactively and proactively), although such effects were only evident under conditions of low biological significance (see Matute & Pineño, 1998, for related work in humans). In the only study to date that has assessed similar interactions in the taste aversion preparation, Turenne, Miles, Parker, and Siegel (1996) reported that when animals received aversion training with morphine prior to place preference conditioning with the same drug, the strength of a conditioned taste aversion was not a significant predictor of the subsequent strength of the place preferences. However, that study included no control group that had not experienced the aversion training prior to the place preference procedure, making it difficult to determine whether the acquired place preferences would have been different had the taste aversion preparation not been given beforehand. To further explore this issue, in Experiment 1 we exposed animals to a place preference procedure with morphine after they had experienced conditioned taste aversion training (also with morphine). Their rates of acquisition and degrees of morphine-induced conditioned place preference were compared to those in groups without a taste aversion history.

Experiment 1

Method

Subjects

The subjects were 102 experimentally naïve, adult male Sprague Dawley rats, purchased from Harlan Sprague Dawley Laboratories (Indianapolis, IN). The animals were delivered to the laboratory at approximately 21 days of age and 42 g. They were maintained under ad libitum food and water until they were approximately 90 days of age and 300 g, at which point the experimental procedures were initiated (see below). The procedures recommended by the National Research Council (1996), the Committee on Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003), and the Institutional Animal Care and Use Committee at American University were followed at all times.

Apparatus

Throughout the conduct of the research, all subjects were individually housed in hanging wire-mesh cages (25.4 × 20 × 18.4 cm), on the front of which graduated Nalgene tubes could be placed for fluid presentation. The subjects were maintained on a 12:12 light:dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C. The place-conditioning apparatus (San Diego Instruments Place Preference System, San Diego, CA) consisted of two main conditioning chambers (28 × 21 × 34.5 cm) joined by a smaller middle chamber (14 × 21 × 34.5 cm). One of the conditioning chambers featured a white aluminum diamond plate floor with white walls; the other conditioning chamber featured a hair-cell-textured black plastic floor with black walls; the smaller middle chamber was outfitted with a steel rod floor and gray walls. Each individual chamber in each apparatus had its own white LED lights, and the lights were set on minimum. The room in which the chambers were located was illuminated by a 25-W red light mounted to the ceiling, and a white noise generator was used to mask background noise. A total of eight identical apparatuses were used; each apparatus featured a 16 × 4 photo-beam array for recording time (in seconds) in each chamber.

Drugs

Morphine sulfate (generously supplied by NIDA) was dissolved in sterile isotonic saline (0.9 %) at a concentration of 5 mg/ml and administered subcutaneously (sc) at a dose of 5 mg/kg. Saccharin (0.1 % sodium saccharin, Sigma Chemical Co.) was prepared as a 1 g/L solution in tap water. All drug weights are expressed as the salt form.

Procedure

Phase 1: Habituation On Day 1 of this phase, subjects were water deprived. Following 23 2/3 h of water deprivation, they were given 20-min access to tap water daily. This procedure was repeated until consumption stabilized—that is, subjects approached and drank from the tube within 2 s of its presentation, and water consumption was within 2 ml of

that from the previous day for a minimum of four consecutive days with no consistent increase or decrease.

Phase 2: Taste aversion conditioning Once consumption stabilized, animals were ranked on average water consumption over the last 3 days of habituation and assigned to one of two groups [i.e., Group S (*n* = 51) and Group W (*n* = 51)], such that consumption was comparable between the groups. On Day 1 of this phase, subjects in Group S were given access to a novel saccharin solution in place of water during their regular 20-min fluid access. Immediately following this presentation, animals in this group were ranked on fluid consumption and assigned to one of two treatment groups (*n* = 25/26), such that the overall consumption was comparable between groups. Approximately 5 min after fluid access, subjects in Group S-M received an sc injection of morphine (5 mg/kg), whereas Group S-V received equivolume saline. Subjects in Group W were given 20-min access to water on this day, assigned to one of two groups (W-M and W-V) on the basis of water consumption, and given an sc injection of 5 mg/kg morphine or equivolume saline, respectively, 5 h after fluid access. The latter procedure was used to give subjects comparable exposure to the injection/drug without an aversion history—that is, administering morphine 5 h after water access is not a condition sufficient to establish a taste aversion (see Freeman & Riley, 2005; Riley, Jacobs, & Mastropaolo, 1983; Lubow, 2009). On the following 3 days (water recovery), all groups were given 20-min access to water (no injections followed this access). This alternating procedure of conditioning and water recovery was repeated for a total of five complete cycles.

Phase 3: Place preference conditioning On Day 1 of this phase (the day after the third water-recovery day of the fifth conditioning cycle; see above), all subjects were given free access to a place preference chamber for 15 min in order to assess initial side preference—that is, the relative time spent in each compartment. A paired-samples *t* test revealed no significant preference for either side [mean time: white → 300.71 s, black → 330.17 s; *t*(101) = −1.788, *p* > .05]. Thus, during subsequent place preference conditioning, an unbiased training procedure (see Cunningham, Ferree, & Howard, 2003; Roma & Riley, 2005) was used, during which the drug was randomly associated with either the black or the white side. On the first place preference conditioning session, subjects were injected (sc) with either morphine (5 mg/kg) or equivolume saline (counterbalanced within each group) and placed in one chamber of the conditioning apparatus (drug-paired side, DPS) for 30 min. This yielded eight groups: specifically, Groups S-M/M (*n* = 14), S-M/V (*n* = 12), S-V/M (*n* = 13), S-V/V (*n* = 12), W-M/M (*n* = 14), W-M/V (*n* = 12), W-V/M (*n* = 13), and W-V/V (*n* = 12), where S or W refers to the fluid given during taste aversion

conditioning (saccharin or water), the first M or V refers to the injection (morphine or vehicle) given during taste aversion conditioning, and the second M or V refers to the injection given during place preference conditioning. On the following day, all animals were injected with equivolume saline and placed in the opposite chamber (nondrug paired side, NDPS) for 30 min. This 2-day cycle was then repeated. On the fifth day (Test 1), all animals were placed in the place preference apparatus for 15 min and allowed to explore both compartments. The time spent in each compartment was recorded for each animal. On the day following this test, all animals were again injected with either morphine (5 mg/kg) or equivolume saline and placed in the DPS of the conditioning apparatus for 30 min. On the following day, they were injected with equivolume saline and placed in the NDPS for 30 min. This 2-day cycle was again repeated and followed on the next day by a second place preference test (Test 2), in which all animals were placed in the place preference apparatus for 15 min and allowed to explore both compartments. As above, the time spent in each compartment was recorded for each animal.

Statistical analyses

During taste aversion conditioning, differences in fluid consumption among the groups were assessed with a 2 × 2 × 5 mixed model analysis of variance (ANOVA), with the between-subjects variables preexposure fluid (saccharin or water) and preexposure drug (morphine or vehicle) and the within-subjects variable trial (1–5). One-way ANOVAs were run for each trial, followed by Tukey's post-hoc analyses to assess differences in consumption among the groups. To evaluate any within-subjects differences across trials, paired-samples *t* tests with Bonferroni corrections were run for each group, comparing the baseline fluid consumption to consumption on each subsequent trial (*p* ≤ .05/16, or .003125). During place preference conditioning, the time (in seconds) spent on the DPS on Pretest, Test 1, and Test 2 was analyzed using a 4 × 2 × 3 mixed model ANOVA with the between-subjects variables aversion history (S-M, S-V, W-M, and W-V) and CPP drug (morphine or vehicle), and the within-subjects variable test (pretest, Test 1, and Test 2). Subsequent one-way ANOVAs were run for each test in order to assess differences in time spent on the DPS among groups. All significance levels were set at *p* ≤ .05.

Results

Phase 2: Taste aversion conditioning

Figure 1 illustrates saccharin (Groups S-M and S-V) or water (Groups W-M and W-V) consumption for all groups over the

five conditioning trials in Phase 2. As is illustrated, animals receiving saccharin paired with morphine acquired significant aversions relative to all other groups and to their own baseline. The $2 \times 2 \times 5$ mixed measures ANOVA on fluid consumption revealed effects of preexposure fluid [$F(1, 98) = 18.865$, $p < .001$], preexposure drug [$F(1, 98) = 101.733$ $p < .001$], and trial [$F(4, 392) = 11.134$, $p < .001$], as well as significant Preexposure Drug $\times$ Trial [$F(4, 392) = 36.561$, $p < .001$], Preexposure Fluid $\times$ Preexposure Drug [$F(1, 98) = 107.471$, $p < .001$], and Preexposure Fluid $\times$ Preexposure Drug $\times$ Trial [$F(4, 392) = 39.043$, $p < .001$] interactions. In relation to the significant Preexposure Fluid $\times$ Preexposure Drug $\times$ Trial interaction, Tukey's post-hoc analyses revealed that Groups S-M and S-V differed significantly in baseline saccharin consumption on Trial 1, $p = .045$. We found no differences among Groups S-V, W-M, and W-V on this trial. On all subsequent trials, Group S-M drank significantly less than all other groups (all ps $\leq .05$). Furthermore, Group S-V drank significantly more fluid than Groups W-M and W-V (all ps $\leq .05$). Groups W-M and W-V did not differ from each other on any trial (all ps $> .05$).

Paired-samples t tests revealed that Group S-M significantly decreased saccharin consumption from baseline (Trial 1) on all subsequent trials (all ps $\leq .003125$). Group S-V significantly increased consumption from baseline (Trial 1) on all subsequent trials (all ps $\leq .003125$). Groups W-M and W-V showed no significant changes in water consumption over conditioning (all ps $> .003125$).

Phase 3: Place preference test

Figure 2 illustrates the time, in seconds, spent on the drug-paired side for each group during the baseline test, as well as any changes in this time from Test 1 to Test 2. As is illustrated, independent of aversion history, animals injected with morphine during place preference conditioning significantly increased their time spent on the DPS, indicating that place
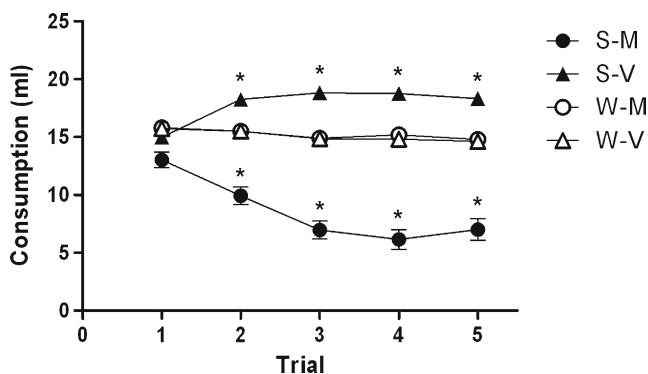

Fig. 1 Experiment 1: Mean ($\pm$ *SEM*) fluid consumption (in milliliters) for each group during Phase 2 (taste aversion conditioning). Significant within-group changes from baseline are noted by asterisks

preferences were acquired. Furthermore, these increases were comparable for all groups. The $4 \times 2 \times 3$ mixed-model ANOVA on time spent on the DPS revealed significant effects of CPP drug [$F(1, 94) = 44.377$, $p < .001$] and test [$F(2, 188) = 39.07$, $p < .001$], as well as a significant CPP Drug $\times$ Test interaction [$F(2, 188) = 35.571$, $p < .001$]. In relation to the significant CPP Drug $\times$ Test interaction, subsequent one-way ANOVAs were run on each test (pretest, Test 1, and Test 2) to assess specific group differences. On the pretest, we observed no significant effect of CPP drug (collapsed across aversion histories) [$F(7, 101) = 1.089$, $p = .377$]. Significant differences were evident on Test 1 and Test 2. Specifically, on Test 1, animals injected with morphine during place preference conditioning spent significantly greater time on the DPS than did animals injected with vehicle [$F(7, 101) = 6.255$, $p < .001$]. This difference was also noted on Test 2 [$F(7, 101) = 11.799$, $p < .001$]. Although morphine induced place preferences, no significant effect emerged of aversion history [$F(3, 94) = 2.265$, $p > .05$], nor any interaction with this term as a factor (all Fs < 3, all ps $> .05$), indicating that a history with taste aversions did not affect the rate or strength of place preference acquisition.

Discussion

To assess whether a history of taste aversion learning with morphine would impact the subsequent acquisition of morphine-induced place preference conditioning, animals in the present experiment were given pairings of saccharin with morphine (taste aversion training) immediately prior to place preference conditioning (with morphine). The data from these animals were compared to data from various controls matched in taste or drug experience, but without the aversion training history. If morphine has both aversive and rewarding effects, but the response conditioned is a function of the specific stimuli with which morphine is paired, both taste aversions and place preferences should be evident. As we reported, taste aversion learning did not impact the ability of morphine to condition preferences; that is, animals displayed both conditioned responses.

The failure of aversion conditioning to affect the acquisition of place preferences was not a simple function of the inability of the specific procedures utilized in the present experiment to induce such effects. First, animals given repeated pairings of saccharin and morphine acquired robust taste aversions, significantly decreasing their saccharin consumption relative to controls, and to their own baseline. Second, all groups given morphine during place preference conditioning acquired place preferences relative to vehicle-treated controls. The fact that a history of aversion learning did not impact place preference conditioning argues instead that morphine has multiple effects and that the specific stimuli present during conditioning are selectively associated
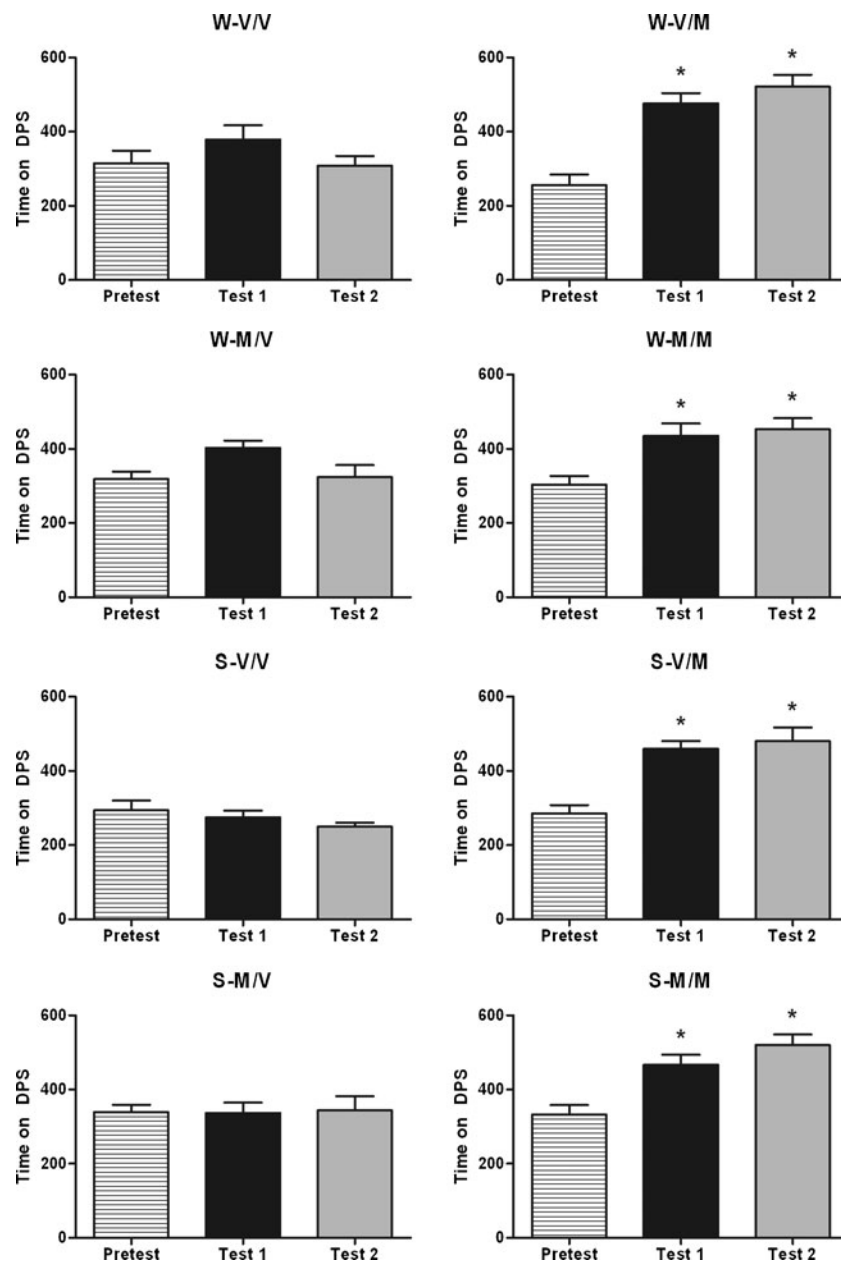
Fig. 2 Experiment 1: Mean time (± *SEM*) spent on the drug-paired side (DPS) for all groups during Phase 3 (place preference conditioning). Significant within-group changes from baseline are noted by asterisks

with these effects (aversive and rewarding) and differentially control avoidance or preference.

Although this interpretation is possible, it is important to note that the findings in Experiment 1 were similar procedurally to others in associative learning, whereby a sequential history with one unique association (A→X) has no impact on a second association in which the same US is given—that is, B → X (Domjan & Burkhard, 1982). In such work, there is no interference between the two associations, given that the associative strength of the US is accrued to each stimulus and to the same degree. Accordingly, the failure of morphine aversion conditioning to affect place

preference conditioning with morphine might be expected, and not be a function of its dual affective properties being selectively associated with specific taste and environmental stimuli.

If the failure of aversion conditioning to affect place preference conditioning in Experiment 1 was a function of processes operating in traditional associative conditioning (see above), it might be expected that place preference conditioning with morphine would be impacted (i.e., blocked) if such conditioning were attempted in the presence of a taste previously associated with that same morphine (Westbrook & Brookes, 1988). Under this condition, no added information

would be provided by the redundant place cues. On the other hand, if morphine has multiple stimulus effects, each of which is selectively associated with specific stimuli, a taste aversion conditioning history with morphine should have no effect on subsequent morphine-induced place preference conditioning, even when such conditioning occurred in the presence of the previously conditioned taste cues. These predictions were tested in Experiment 2, in which a modified blocking design was used to examine the interaction of taste aversion and place preference conditioning with morphine.

Experiment 2

Method

Subjects, apparatus, and drug

The subjects were 32 rats of the same age, strain, and sex that were used in the previous experiment. They were maintained under conditions comparable to those previously described. Morphine sulfate was prepared and administered as in Experiment 1.

Procedure

Phase 2: Taste aversion conditioning Following habituation (Phase 1; see above), animals were given 20-min access to a novel saccharin solution. On the basis of saccharin consumption, they were divided into two groups—that is, Group M ($n = 16$) and Group V ($n = 16$)—such that consumption was comparable between the groups, and then were injected sc with either morphine sulfate or equivolume saline (approximately 5 min after fluid access). This trial was followed by three water-recovery days. This procedure was repeated for a total of five complete cycles.

Phase 3: Place preference conditioning All subjects were then given a pretest in the place preference chambers to determine side preferences. A paired-samples t test revealed significant differences in side preference [mean times: white → 264.2 s, black → 336.65 s; $t(31) = -2.495$, $p = .018$]. Accordingly, a biased training procedure was used, during which the drug was associated with the side on which each animal spent less time during pretest. On the first place preference conditioning session, subjects within each group (M and V) were assigned (counterbalanced across groups) to receive either saccharin or water during their regular 20-min fluid access. This yielded four groups, M-S, M-W, V-S, and V-W (the first letter referring to the drug given during taste aversion conditioning, and the second letter referring to the taste cue given during place preference conditioning). Immediately after fluid access (saccharin or water), subjects

were injected sc with morphine (5 mg/kg) and placed in the nonpreferred chamber of the conditioning apparatus (drug-paired side, DPS) for 30 min. On the following day, all animals were given water during their fluid access period and then injected with equivolume saline and placed in the opposite chamber (non-drug-paired side, NDPS) for 30 min. This 2-day cycle was then repeated, and a test of place preferences was conducted as described above.

Statistical analyses

During taste aversion conditioning, differences in fluid consumption among the groups were assessed with a 2×5 mixed model ANOVA with the between-subjects variable conditioning drug (morphine or vehicle) and the within subjects variable trial (1–5). One-way ANOVAs were run for each trial in order to assess differences in consumption among the groups. To evaluate any within-subjects differences across trials, paired-samples t tests with Bonferroni corrections were run for each group, comparing baseline fluid consumption to the consumption on each subsequent trial ($p \leq .05/8$, or .00625). During place preference conditioning, the time (in seconds) spent on the DPS during pretest and test was analyzed using a $2 \times 2 \times 2$ mixed model ANOVA with the between-subjects variables conditioning drug (morphine or vehicle) and taste solution given during CPP training (saccharin or water), and the within-subjects variable trial (pretest or test). All significance levels were set at $p \leq .05$.

Results

Phase 2: Taste aversion conditioning

Figure 3 illustrates the fluid consumption for Groups M and V over the five conditioning trials in Phase 2. As is illustrated, the animals receiving saccharin paired with morphine acquired significant aversions, relative to vehicle controls and to their own baseline. The 2×5 mixed-model ANOVA on fluid consumption revealed an effect of conditioning drug [$F(1, 30) = 76.47$, $p < .001$], as well as a significant Conditioning Drug $\times$ Trial interaction [$F(4, 120) = 62.629$, $p < .001$], but no effect of trial [$F(4, 120) = 1.779$, $p = .137$]. In relation to the significant Conditioning Drug $\times$ Trial interaction, a one-way ANOVA revealed that Groups M and V did not differ significantly in baseline saccharin consumption on Trial 1 [$F(1, 31) = .094$, $p = .761$]. On all subsequent trials, Group M drank significantly less than Group V (all ps $\leq .01$), indicating the acquisition of an aversion to the morphine-associated solution.

Paired-samples t tests revealed that Group M significantly decreased its saccharin consumption from baseline (Trial 1)
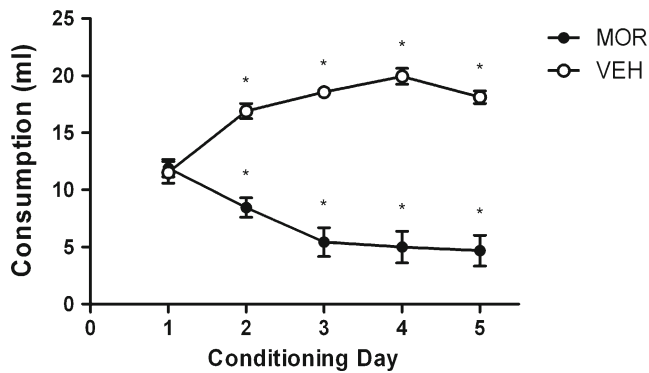
Fig. 3 Experiment 2: Mean (± *SEM*) fluid consumption (in milliliters) for each group during Phase 2 (taste aversion conditioning). Significant within-group changes from baseline are noted by asterisks

Fig. 4 Experiment 2: Mean time (± *SEM*) spent on the drug-paired side (DPS) for all groups during Phase 3 (combined taste aversion/place preference conditioning). Significant within-group changes from baseline are noted by asterisks

on all subsequent trials (all $ps ≤ .00625$). Group V, on the other hand, significantly increased its consumption from baseline (Trial 1) on all subsequent trials (all $ps ≤ .00625$).

Phase 3: Place preference test

Figure 4 illustrates the time, in seconds, spent on the drug-paired side for each group during the baseline test, as well as any changes in this time from pretest to test. As is illustrated, independent of aversion history or the type of solution provided immediately prior to place preference conditioning, animals injected with morphine during place preference conditioning significantly increased their time spent on the DPS, indicating that place preferences were acquired. Furthermore, these increases were comparable for all groups. The $2 × 2 × 2$ mixed-model ANOVA on time spent on the DPS revealed a significant effect of trial [$F(1, 28) = 49.58$, $p < .001$], but no effects of conditioning drug or taste solution, nor any significant interaction with either of these two terms as a factor (all $Fs < 2$, all $ps > .05$). In relation to the significant effect of trial, all groups increased their time spent on the DPS from pretest to test, indicating that comparable place preferences were acquired by all groups.

Table 1 presents the amounts of saccharin consumed on the final test day during taste aversion conditioning in Phase 2, as well as the amount of saccharin (Groups M-S and V-S) or water (Groups M-W and V-W) consumed during place preference training in Phase 3. These data reveal that subjects given aversion training and then access to saccharin during place preference conditioning (Group M-S) continued to avoid the saccharin solution, indicative of the maintenance of the aversion. Control subjects given access to saccharin during place preference conditioning (Group V-S) drank at high levels. Finally, subjects given access to water during place preference conditioning—that is, Groups M-W and V-W—drank water at high levels, indicating that water consumption was unaffected by taste aversion history.
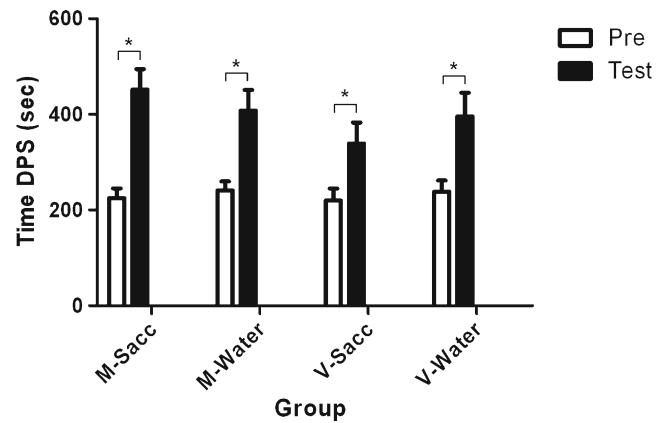
Discussion

In Experiment 2, animals with a history of taste aversion conditioning with morphine were assessed for their ability to acquire morphine-induced place preferences in the context of the morphine-associated taste cue. As described, despite being presented along with the aversive taste, conditioned place preferences were established in this group, and these preferences did not differ from groups without this prior conditioning history or with this history but conditioned in the absence of the taste cue. These results are consistent with the position that morphine has multiple stimulus effects (aversive and rewarding), each of which is selectively associated with specific stimuli (taste and environmental cues) that differentially control behavior (taste aversions and place preferences).

The failure to see blocking in this assessment was not due to the fact that such effects are not evident in the taste aversion preparation. As early as 1971, Revusky reported that a flavored solution (e.g., coffee) that had previously been paired with LiCl (thus conditioning an aversion) subsequently blocked the acquisition of an aversion to a saccharin solution when it was given between the pairing of saccharin and LiCl

Table 1 Amounts of saccharin consumed (in milliliters) on the final test day during taste aversion conditioning in Phase 2, as well as the amounts of saccharin (Groups M-S and V-S) or water (Groups M-W and V-W) consumed (in milliliters) during place preference training in Phase 3

	M-S		M-W		V-S		V-W	
	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
CTA Test	3.56	1.45	5.81	2.30	18.25	0.92	18.00	0.67
DPS1	3.62	1.61	15.00	0.66	18.75	0.90	15.12	0.63
DPS2	2.75	1.43	14.31	0.71	15.25	0.74	13.87	0.46

(see also Domjan & Gillan, 1977). Blocking has also been reported across stimulus modalities. For example, animals injected with LiCl after being placed in a novel chamber subsequently fail to acquire aversions to a novel saccharin solution when the saccharin–LiCl pairing was given in that environment. That is, the LiCl-associated environment blocks the acquisition of the saccharin–LiCl association (for a summary of such findings, see Batson & Best, 1979; for a review of US preexposure, see Riley & Simpson, 2001).

Although the abovementioned findings argue that blocking is evident in the taste aversion preparation (even across stimulus modalities), it is important to note several differences between those designs and the one used in our Experiment 2. First, these demonstrations used LiCl as the conditioning agent, whereas morphine was used in the present series of experiments. Although a history with morphine has been reported to attenuate the subsequent acquisition of morphine-induced taste aversions (see Hunt, Spivak, & Amit, 1985; LeBlanc & Cappell, 1974; Simpson & Riley, 2005), there is little evidence that such attenuating effects are associative in nature; that is, they are reported independent of the similarity of the preexposure and conditioning environments, suggesting that such effects are nonassociative in nature (see Domjan & Siegel, 1983; Riley, Dacanay, & Mastropaolo, 1984; Stewart & Eikelboom, 1978). Second, the specific procedure utilized here is a modified blocking design in which the taste stimulus previously associated with morphine was not given concurrent with the environment in the second phase of the study. In the design utilized here, the morphine-associated taste was given 20 min prior to the animal's placement in the place preference conditioning apparatus. Although in more traditional blocking designs the two stimuli have been presented concurrently during conditioning, blocking has been reported when the two conditioned stimuli (CSs) are presented in a serial manner (see Kehoe, Schreurs, & Amodei, 1981; Kohler & Ayres, 1982; Solomon, 1977), even in the taste aversion procedure (Revusky, 1971). Third, morphine was given prior to placement of the animals in the place-conditioning apparatus. In the more traditional procedures, the design is A→X and then AB→X, whereas the present design was A→X and then A→X→B. A closer approximation to the blocking procedure would have had the animals being given saccharin in the place preference apparatus prior to the injection of morphine. The design used in the present experiment was chosen because the ability of some drugs of abuse to condition place aversions or preferences is dependent on the specific temporal parameters used during conditioning. For example, mice given alcohol after placement in a place preference chamber display conditioned place aversions. Place preferences are conditioned only if the drug is given prior to the placement in the apparatus (Cunningham, Okorn, & Howard, 1997; Cunningham, Tull, Rindal, & Meyer, 2002). It has been argued

that if the animal is in the place preference chamber during the onset of the drug's aversive effects, place aversions would be conditioned and control behavior. By delaying placement into the chamber, the animal is in the apparatus as the drug's rewarding effects occur. Although such an assessment has not been directly tested with morphine (and may be drug-dependent; see Ettenberg, Raven, Danluck, & Necessary, 1999), the procedure used here was chosen to circumvent any possible temporal effects of the compound that might have impacted conditioning. It remains unknown to what extent the temporal effects of morphine contribute to the differential control exerted by taste and place stimuli, and if the selective effects—that is, taste→aversive and place→rewarding—that we report are a function of the timing of the affective response.

Although the failure of blocking may be impacted by the abovementioned procedural issues, it is interesting that similar data have been presented for other stimuli with apparent multiple properties. For example, Betts, Brandon, and Wagner (1996) assessed the ability of a CS associated with paraorbital shock to block subsequent conditioning to another CS paired with the same shock, but with the locus of the shock having changed (i.e., from one eye to the other). Interestingly, blocking was dependent upon the specific measure used in the assessment. That is, when startle was used as the conditioned response, blocking was found. However, when the eyeblink itself was the measure, blocking was not evident. It was concluded that these data were a function of the fact that paraorbital shock has "multiple nodes in the memory system" (Konorski, 1967)—specifically, emotional and sensory. The emotional component (which mediates the startle response to the shock) was unchanged with the specific location of the shock. Accordingly, when conditioning with a second CS was attempted in the presence of the previously conditioned stimulus, no change was observed in this component, thus allowing blocking to be seen. The sensory component of the shock, however, varied upon the specific eye receiving the shock. Under the shifted condition, the sensory experience was different, preventing blocking. Although the design of the present experiments was quite different from that of Betts et al., if the affective (emotional) responses to morphine are multifaceted (both rewarding and aversive) and dependent upon the specific stimuli present, the two stimuli (in this case, taste and place) become differentially associated with the multiple effects of morphine, again precluding blocking.

Although the basis for the present data is not known, what is clear is that a history of taste aversion conditioning (with morphine) has no effect on morphine's ability to induce a place preference. This is evident when place preference conditioning occurs in the absence (Exp. 1) or presence (Exp. 2) of the taste previously associated with morphine. Although several issues remain unresolved (see above), the

data in the present study are consistent with the position that drugs of abuse have multiple stimulus effects (positive and negative) that are differentially associated with specific stimulus events that direct approach and avoidance (see Hunt & Amit, 1987; Riley, 2011; Stolerman, 1992). The fact that such drugs are complex stimuli makes them somewhat unique in controlling behavior, and understanding these multiple properties provides insight into the selective behaviors that they condition and how these multiple properties do and do not interact (see also Verendeev & Riley, 2012).

References

Batson, J. D., & Best, P. J. (1979). Drug-preexposure effects in flavor-aversion learning: Associative interference by conditioned environmental stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 5,* 273–283. doi:10.1037/0097-7403.5.3.273

Bechara, A., & Van der Kooy, D. (1985). Opposite motivational effects of endogenous opioids in brain and periphery. *Nature, 314,* 533–534. doi:10.1038/314533a0

Betts, S. L., Brandon, S. E., & Wagner, A. R. (1996). Dissociation of the blocking of conditioned eyeblink and conditioned fear following a shift in US locus. *Animal Learning & Behavior, 24,* 459–470.

Cappell, H., & LeBlanc, A. (1971). Conditioned aversion to saccharin by single administrations of mescaline and *d*-amphetamine. *Psychopharmacology, 22,* 352–356. doi:10.1007/BF00406873

Cappell, H., & Le Blanc, A. (1975). Conditioned aversion by psychoactive drugs: Does it have significance for an understanding of drug dependence? *Addictive Behaviors, 1,* 55–64. doi:10.1016/S0306-4603(75)80018-9

Cappell, H., LeBlanc, A., & Endrenyi, L. (1973). Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacology, 29,* 239–246. doi:10.1007/BF00414038

Cunningham, C. L., Ferree, N. K., & Howard, M. K. A. (2003). Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology, 170,* 409–422.

Cunningham, C. L., Okorn, D. M., & Howard, C. E. (1997). Interstimulus interval determines whether ethanol produces conditioned place preference or aversion in mice. *Animal Learning & Behavior, 25,* 31–42.

Cunningham, C. L., Tull, L. E., Rindal, K. E., & Meyer, P. J. (2002). Distal and proximal pre-exposure to ethanol in the place conditioning task: Tolerance to aversive effect, sensitization to activating effect, but no change in rewarding effect. *Psychopharmacology, 160,* 414–424.

Domjan, M., & Burkhard, B. (1982). *The principles of learning and behavior*. Belmont, CA: Wadsworth, Inc.

Domjan, M., & Gillan, D. J. (1977). Aftereffects of lithium-conditioned stimuli on consummatory behavior. *Journal of Experimental Psychology: Animal Behavior Processes, 3,* 322–334. doi:10.1037/0097-7403.3.4.322

Domjan, M., & Siegel, S. (1983). Attenuation of the aversive and analgesic effects of morphine by repeated administration: Different mechanisms. *Physiological Psychology, 11,* 155–158.

Elsmore, T. F., & Fletcher, G. V. (1972). Delta 9-tetrahydrocannabinol: Aversive effects in rat at high doses. *Science, 175,* 911–912. doi:10.1126/science.175.4024.911

Escobar, M., Matute, H., & Miller, R. R. (2001). Cues trained apart compete for behavioral control in rats: Convergence with the associative interference literature. *Journal of Experimental Psychology: General, 130,* 97–115. doi:10.1037/0096-3445.130.1.97

Ettenberg, A., Raven, M. A., Danluck, D. A., & Necessary, B. D. (1999). Evidence for opponent-process actions of intravenous cocaine. *Pharmacology Biochemistry and Behavior, 64,* 507–512.

Freeman, K. B., & Riley, A. L. (2005). Cocaine-induced conditioned taste avoidance over extended conditioned stimulus–unconditioned stimulus intervals. *Behavioural Pharmacology, 16,* 591–595.

Freeman, K. B., & Riley, A. L. (2009). Origins of CTA Learning. In S. Reilly & T. R. Schachtman (Eds.), *Conditioned taste aversion: Behavioral and neural processes* (pp. 9–33). Oxford, UK: Oxford University Press.

Garcia, J., & Ervin, F. (1968). Gustatory-visceral and telereceptor-cutaneous conditioning: Adaptation in internal and external milieus. *Communications in Behavioral Biology, Part A, 1,* 389–415.

Garcia, J., Kimeldorf, D., & Koelling, R. (1955). Conditioned aversion to saccharin resulting from exposure to gamma radiation. *Science, 122,* 157–158.

Garcia, J., & Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic Science, 4,* 123–124.

Garcia, J., & Koelling, R. A. (1967). A comparison of aversions induced by X rays, toxins, and drugs in the rat. *Radiation Research, 7,* 439–450. doi:10.2307/3583736

Garcia, J., McGowan, B., Ervin, F., & Koelling, R. (1968). Cues: Their relative effectiveness as a function of the reinforcer. *Science, 160,* 794–795. doi:10.1126/science.160.3829.794

Goudie, A. (1979). Aversive stimulus properties of drugs. *Neuropharmacology, 18,* 971–979.

Grigson, P. S. (1997). Conditioned taste aversions and drugs of abuse: A reinterpretation. *Behavioral Neuroscience, 111,* 129–136. doi:10.1037/0735-7044.111.1.129

Hunt, T., & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: Paradox revisited. *Neuroscience and Biobehavioral Reviews, 11,* 107–130. doi:10.1016/S0149-7634(87)80005-2

Hunt, T., Spivak, K., & Amit, Z. (1985). Aversive stimulus properties of morphine: evaluation using the drug preexposure conditioned taste aversion paradigm. *Behavioral and Neural Biology, 44,* 60–73.

Kehoe, E. J., Schreurs, B., & Amodei, N. (1981). Blocking acquisition of the rabbit's nictitating membrane response to serial conditioned stimuli. *Learning and Motivation, 12,* 92–108.

Kohler, E. A., & Ayres, J. J. (1982). Blocking with serial and simultaneous compounds in a trace conditioning procedure. *Animal Learning & Behavior, 10,* 277–287.

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.

LeBlanc, A., & Cappell, H. (1974). Attenuation of punishing effects of morphine and amphetamine by chronic prior treatment. *Journal of Comparative and Physiological Psychology, 87,* 691–698. doi:10.1037/h0036978

Lester, D., Nachman, M., & Le Magnen, J. (1970). Aversive conditioning by ethanol in the rat. *Quarterly Journal of Studies on Alcohol, 31,* 578–586.

Lubow, R. E. (2009). Conditioned taste aversion and latent inhibition: A review. In S. Reilly & T. R. Schachtman (Eds.), *Conditioned taste aversion: Behavioral and neural processes* (pp. 37–57). Oxford: Oxford University Press.

Martin, J. C., & Ellinwood, E. H. (1973). Conditioned aversion to a preferred solution following methamphetamine injections. *Psychopharmacology, 29,* 253–261. doi:10.1007/BF00414040

Matute, H., & Pineño, O. (1998). Stimulus competition in the absence of compound conditioning. *Animal Learning & Behavior, 26,* 3–14.

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press.

National Research Council (2003). *Guidelines for the Care and Use of Mammals in Neuroscienceand Behavioral Research*. Washington, DC: National Academy Press.

Revusky, S. (1971). The role of interference in association over a delay. In W. Honig (Ed.), *Animal memory* (pp. 155–213). New York: Academic Press.

Riley, A. L. (2011). The paradox of drug taking: The role of the aversive effects of drugs. *Physiology and Behavior, 103,* 69–78.

Riley, A. L., Dacanay, R. J., & Mastropaolo, J. P. (1984). The effect of morphine preexposure on the acquisition of morphine-induced taste aversions: A nonassociative effect. *Animal Learning & Behavior, 12,* 157–162.

Riley, A. L., Jacobs, W. J., & Mastropaolo, J. P. (1983). The effects of extensive taste preexposure on the acquisition of conditioned taste aversions. *Bulletin of the Psychonomic Society, 21,* 221–224.

Riley, A. L., & Simpson, G. R. (2001). The attenuating effects of drug preexposure on taste aversion conditioning: Generality, experimental parameters, underlying mechanisms, and implications for drug use and abuse. In R. R. Mowrer & S. B. Klein (Eds.), *Handbook of contemporary learning theories* (pp. 505–559). Mahwah: Erlbaum.

Roma, P. G., & Riley, A. L. (2005). Apparatus bias and the use of light and texture in place conditioning. *Pharmacology Biochemistry and Behavior, 82,* 163–169.

Simpson, G. R., & Riley, A. L. (2005). Morphine preexposure facilitates morphine place preference and attenuates morphine taste aversion. *Pharmacology Biochemistry and Behavior, 80,* 471–479. doi:10.1016/j.pbb.2005.01.003

Solomon, P. R. (1977). Role of the hippocampus in blocking and conditioned inhibition of the rabbit's nictitating membrane response. *Journal of Comparative and Physiological Psychology, 91,* 407–417.

Stewart, J., & Eikelboom, R. (1978). Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. *Pharmacology Biochemistry and Behavior, 9,* 639–645.

Stolerman, I. (1992). Drugs of abuse: Behavioural principles, methods and terms. *Trends in Pharmacological Sciences, 13,* 170–176.

Turenne, S. D., Miles, C., Parker, L. A., & Siegel, S. (1996). Individual differences in reactivity to the rewarding/aversive properties of drugs: Assessment by taste and place conditioning. *Pharmacology Biochemistry and Behavior, 53,* 511–516.

Verendeev, A., & Riley, A. L. (2012). Conditioned taste aversion and drugs of abuse: History and interpretation. *Neuroscience and Biobehavioral Reviews, 36,* 2193–2205.

Westbrook, R., & Brookes, N. (1988). Potentiation and blocking of conditioned flavour and context aversions. *Quarterly Journal of Experimental Psychology, 40,* 3–30.

White, N., Sklar, L., & Amit, Z. (1977). The reinforcing action of morphine and its paradoxical side effect. *Psychopharmacology, 52,* 63–66. doi:10.1007/BF00426601

Wise, R. A., Yokel, R. A., & DeWitt, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science, 191,* 1273–1275. doi:10.1126/science.1257748