

Reduced Palatability in Drug-Induced Taste Aversion: II.  
Aversive and Rewarding Unconditioned Stimuli

Joe Arthurs, Jian-You Lin, Leslie Renee Amodeo and Steve Reilly

University of Illinois at Chicago

Correspondence: Jian-You Lin or Steve Reilly  
Department of Psychology  
University of Illinois at Chicago  
1007 West Harrison Street  
Chicago, IL 60607  
  
Fax: (312) 413-4122  
Email: [jlin2@uic.edu](mailto:jlin2@uic.edu) or [sreilly@uic.edu](mailto:sreilly@uic.edu)

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### Abstract

Drugs of abuse are known to reduce intake of a taste conditioned stimulus (CS), a behavioral response sometimes seen as paradoxical because the same drugs also serve as rewards in other behavioral procedures. In the present study we compared patterns of intake and palatability (assessed using microstructural analysis of licking) for a standard saccharin CS paired with: lithium chloride, morphine, amphetamine, or sucrose. We found that morphine and amphetamine, like lithium-induced illness, each suppressed CS intake and caused a reduction in saccharin palatability. Sucrose, a rewarding stimulus, did not reduce the palatability of the saccharin CS. We interpret these finds as evidence that drugs of abuse induce conditioned taste aversions.

*Keywords:* microstructure, cluster size, morphine, amphetamine, incentive contrast, rats

From the long perspective of evolutionary history, poisons or toxins only gained entry into the body because they were unknowingly consumed in food. It is not surprising that ingestive defense mechanisms would evolve to protect against such occurrences. One such mechanism, conditioned taste aversion (CTA; for reviews see Barker, Best & Domjan, 1977; Braveman & Bronstein, 1985; Milgram, Krames & Alloway, 1977; Reilly & Schachtman, 2009), prevents the repeated self-administration of a poison or toxin that occurs in a distinctively tasting food. This learning phenomenon involves the development of an association between the particular taste (conditioned stimulus, CS) of the food and the consequent illness (unconditioned stimulus, US) and is manifest in behavior as a reluctance to eat, or complete avoidance of, that food when re-encountered. So powerful is this mechanism that CTA occurs even when the animal is rendered comatose after consumption of the CS (e.g., Bermudez-Rattoni, Forthman, Sanchez, Perez & Garcia, 1988; Roll & Smith, 1972).

CTAs are conditioned by USs that cause gastrointestinal illness. Moreover, the taste CS is not just a cue that signals the occurrence of the US, the taste seems to become disliked or distasteful (Garcia, 1989; Pelchat & Rozin, 1982). That is, the taste per se is devalued as a consequence of its association with illness. Many agents can serve as a US to induce CTAs (Gamzu, Vincent & Boff, 1985; Riley & Tuck, 1985) including self-administered drugs of abuse such as alcohol, amphetamine and morphine (Berger, 1972; Cappell & LeBlanc, 1971; Cappell, LeBlanc & Endrenyi, 1973; Carey, 1973; Davison & House, 1975; Le Magnen, 1969; Nachman, Lester & Le Magnen, 1970; Nathan & Vogel, 1975; Riley, Jacobs & LoLordo, 1978). Drugs of abuse were initially considered to produce CTAs because they caused a reduction in the amount

consumed of the associated taste CS. Some researchers doubted this analysis because unlike the prototypical illness-inducing agents (e.g., motion sickness, radiation, toxic chemicals) that serve as USs, drugs of abuse are self-administered and rewarding. That psychoactive drugs might have aversive and rewarding aspects was viewed as a paradox by some investigators (e.g., Cappell & LeBlanc, 1973; Hunt & Amit, 1987; Vogel & Nathan, 1975). Empirical investigation of this controversial issue benefited greatly from studies that employed taste reactivity methodology (Grill & Norgren, 1978a) to examine the hedonic value or palatability of the taste CS in non-human animals.

Across mammalian species a stereotypical set of orofacial reactions are elicited by controlled infusions of a taste solution into the mouth. Thus, a highly preferred taste such as sucrose evokes a predictable series of mouth movements, tongue protrusions (forward and lateral), followed by face and paw washing. On the other hand, a highly non-preferred solution such as quinine evokes gaping, chin rubbing, headshakes, face washing and paw treading behaviors. Less intense stimuli of either valence elicit a subset of these behaviors (for reviews see Berridge, 2000; Grill & Berridge, 1985). Nowadays, the taste reactivity test is a standard measure widely viewed as an effective technique to directly assess the palatability of taste stimuli. Applying this methodology to determine the nature of CTAs revealed that when an otherwise acceptable taste stimulus is paired with an illness-inducing agent such as lithium chloride (LiCl), ingestive orofacial responses decline and are replaced by aversive or rejection responses. That is, LiCl supported a conditioned reduction in the hedonic value or palatability of the taste CS (Breslin, Spector & Grill, 1992; Ossenkopp & Eckel, 1995; Parker, 1995; Spector, Breslin & Grill, 1988). However, when a drug of abuse is paired with a taste stimulus it is

reported that there is no increase in aversive/rejection taste reactivity responses. This finding, of the absence of a conditioned reduction in taste palatability by a variety of drugs of abuse (Davies & Wellman, 1990; Mayer & Parker, 1993; Parker, 1982, 1988, 1991, 1993, 1996; Parker & Carvell, 1986; Zalaquett & Parker, 1989), is taken as evidence that drug-induced intake suppression is qualitatively different from illness-induced CTAs (e.g., Grigson, 1997; Grigson, Twining, Freet, Wheeler & Geddes, 2009; Parker, 1995, 2003; Parker, Limebeer & Rana, 2009). But, is this conclusion correct?

As discussed in the companion paper (Lin, Arthurs, Amodeo & Reilly, 2012), there are a number of reasons to seek alternative ways to investigate the nature of drug-induced taste learning. One major issue is that the taste reactivity procedure, initially developed for use with decerebrate rats that are incapable of spontaneous behaviors (Grill & Norgren, 1978b), is an unnatural task because it involves ingestion and rejection of stimuli placed directly into the subject's mouth by the experimenter not by the subject. Accordingly, employing a common taste CS (saccharin), the present study evaluated LiCl-induced (Experiment 1) and drug-induced (Experiments 2 and 3) taste learning using our standard voluntary intake procedure and parameters (e.g., Lin, Arthurs & Reilly, 2011; Lin, Roman, St. Andre & Reilly, 2009; Roman, Lin & Reilly, 2009) augmented by a microstructural assessment of the temporal distribution of lick patterns. Using this more fine-grained analysis, the size of a cluster of licks (herein defined as sustained run of licks with interlick intervals less than 0.5 s) is established as a sensitive measure of taste palatability (see, for example, Davis, 1989, 1996, 1998; Davis & Smith, 1992; Hsiao & Fan, 1993; Spector, Klumpp & Kaplan, 1998; Spector & St. John, 1998). In addition, by dividing the total number of licks by volume consumed on each trial we

obtained a measure of lick efficiency (i.e., the number of licks required to obtain 1 g of fluid – the higher this number the smaller the lick volume). Finally, to afford a more complete assessment of conditioned changes in palatability, we also examined (Experiment 4) saccharin suppression induced by consumption of sucrose, generally regarded as one of the best exemplars of a purely rewarding stimulus, using an anticipatory negative contrast procedure (see Flaherty, 1996).

### Experiment 1

Whether assessed with a decrease in volume consumed or an increase in taste reactivity aversive/rejection responses, there is general agreement that LiCl conditions a reduction in the hedonic value or palatability of the associated taste CS. The primary goal of Experiment 1 was to obtain with our methods a pattern of results for a downshift in the palatability of the saccharin CS against which the performance shown with the USs used in Experiments 2-4 might be compared.

### Method

**Subjects.** Sixteen experimentally naïve male Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) served as subjects. They were individually housed in hanging stainless steel cages (Acme Metal Product, Chicago, IL) in a vivarium maintained at 70° on a 12hr:12hr light cycle with lights on at 7:00 am. All experimental treatments and procedures occurred during the light phase of the cycle. Food and water were available in the home cage *ad libitum* except during behavioral testing as noted below. Animals were treated in accordance with guidelines from the

American Psychological Association (1996) and the National Institutes of Health (1996) and the University of Illinois at Chicago Animal Care Committee approved the procedures and protocols employed herein.

**Apparatus.** Eight identical modular drinking chambers (Med Associates, St. Albans, VT) were used. Each chamber (30.5 cm long X 24.0 cm wide X 29.0 cm high) had a clear polycarbonate door, back wall, and ceiling. The triple panel modular sidewalls were made of aluminum and the floor consisted of 19 stainless steel rods. A shaded light bulb (100 mA, 28 V), which reflected light onto the ceiling, and white noise generator (background noise level ~80 dB) were mounted at the top of the middle panel of the left wall. Solutions were presented in a retractable stimulus bottle the tip of which was available through a rounded access hole (1.3 cm wide X 2.6 cm high) centered 6.0 cm above the grid floor in the middle panel of the right wall. In the extended position, the tip of the spout was centered in the access hole ~3 mm outside the chamber to prevent constant contact. A lickometer circuit (0.3  $\mu$ A) monitored licking to within 10 milliseconds. Each drinking chamber was housed within a sound-attenuating cubicle fitted with a ventilation fan. The chambers were connected to a computer in an adjacent room, which controlled all events and recorded data using programs written in the Medstate notation language.

**Procedure.** Animals were allowed to habituate to the facility for 5 days prior to the initiation of any treatments and were handled for 5 min each day. Thereafter, the animals were adapted to a water deprivation schedule that involved two 15-min water access periods per day, one in the morning and the second four hours later in the afternoon. During the first four days of this schedule all water access was available in

the home cage. During the last three of these days the animals were given 15-min per day context habituation to the drinking chambers. On the next day, morning water access was switched in to the drinking chambers until intake and lick patterns stabilized (~8 days). Animals were then counterbalanced for intake, licks, cluster size, and lick efficiency and assigned to two groups according to the injection that would be administered on the conditioning days: saline (1.33 ml/100 g body weight for Group Unpaired;  $n = 8$ ) or LiCl (0.037 M at 1.33 ml/100 g for Group Paired;  $n = 8$ ). Testing was conducted in three-day cycles consisting of a conditioning day, a control day, and a recovery day. On conditioning days animals were presented with 0.15% saccharin for 15 min in place of morning water and injected 30 min later with LiCl (Group Paired) or saline (Group Unpaired). On control days, animals were presented with water for 15 min in the drinking chambers followed 30 min later by an injection of saline (Group Paired) or LiCl (Group Unpaired), and on recovery days animals were given 15-min water access in the drinking chambers with no subsequent injection. This cycle was repeated twice followed by a final taste only test trial.

## **Data Analysis**

Water intake data on the final baseline trial were assessed using an independent  $t$ -test. A mixed model analysis of variance (ANOVA) was conducted for each measure with Group (Unpaired-Paired) as the between-subject variable and Trial as the within-subject variable. A significant main effect or interaction was followed up by post hoc analyses, simple main effects, with the adjusted error term adopted from the overall



ANOVA. The alpha level was set at  $p < .05$ . All these analyses were conducted with Statistica 6.0 software (StatSoft, Inc. Tulsa, OK).

## Results and Discussion

--- INSERT TABLE 1 ABOUT HERE ---

Verifying the counterbalancing, statistical analysis conducted on the water intake data on the day before the first conditioning trial (see Table 1) found no significant difference between the Unpaired and Paired groups for amount consumed, total licks, cluster size, or lick efficiency (all  $t < 1$ ).

--- INSERT FIGURE 1 ABOUT HERE ---

Intake, total licks, clusters size, and lick efficiency for Group Unpaired and Group Paired over the two conditioning trials and the taste only test trial are depicted in Figure 1. Inspection of Figure 1A shows that saccharin intake in the two groups moved in opposite directions during the experiment: Group Unpaired displayed a recovery from the initial neophobia reaction to the novel saccharin taste whereas intake in Group Paired declined across trials as a CTA was acquired. These impressions were confirmed with an ANOVA that found a significant main effect of Group,  $F(1, 14) = 44.04$ ,  $p < .05$ , as well as a significant Group x Trial interaction,  $F(2, 28) = 133.45$ ,  $p < .05$ ; the main effect of Trial was not significant ( $p > .20$ ). Post hoc analysis found no significant group differences on Trial 1 ( $p > .05$ ) but while Group Unpaired significantly increased intake across the next two trials ( $ps < .05$ ) the amount consumed by Group Paired significantly decreased on Trials 2 and 3 ( $ps < .05$ ). For each group, total number of licks for saccharin (Figure 1B) showed a pattern almost identical to amount consumed. An ANOVA conducted on the data summarized in the figure revealed a

significant main effect of Group,  $F(1, 14) = 75.74$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 28) = 64.09$ ,  $p < .05$ . There was, however, no significant main effect of Trial ( $F < 1$ ). Post hoc comparisons revealed an identical pattern of significance ( $ps < .05$ ) as amount consumed except the increase in total licks from Trial 2 to Trial 3 in Group Unpaired narrowly failed to achieve significance ( $p = .053$ ). Analysis of cluster size data (see Figure 1C) revealed a significant main effect of Group,  $F(1, 14) = 79.51$ ,  $p < .05$ , a significant main effect of Trial,  $F(2, 28) = 7.20$ ,  $p < .05$ , and a Group x Trial interaction  $F(2, 28) = 31.80$ ,  $p < .05$ . Follow up statistics found no between-group differences on Trial 1 ( $p > .05$ ). Furthermore, Group Unpaired significantly increased cluster size from Trial 1 to Trial 2 ( $p < .05$ ) and that the increase from Trial 2 to Trial 3 narrowly failed to achieve significance ( $p = .051$ ). On the other hand, Group paired showed a significant reduction in cluster size from Trial 1 to Trial 2 ( $p < .05$ ) but not from Trial 2 to Trial 3 ( $p > .05$ ). Although inspection of the results in Figure 1D suggests that the Paired rats showed a reduction of lick efficiency over trials relative to the performance of the Unpaired subject, the large degree of variance in the data prevented the numerical differences from achieving significance. That is, both the main effect of Group,  $F(1, 14) = 4.58$ ,  $p = .05$ , and the Group X Trial interaction,  $F(2, 28) = 3.06$ ,  $p = .06$ , were only marginally significant.

As expected, LiCl induced a CTA manifest in performance as a significant reduction in volume consumed, total licks and cluster size. This pattern of results is consistent with the few other reports that have used a microstructural analysis of lick patterns to examine LiCl-induced reduction in taste palatability (Baird, St. John & Nguyen, 2005; Dwyer, 2009; Dwyer, Boakes & Hayward, 2008; Kent, Cross-Mellor,

Kavaliers & Ossenkopp, 2002). Providing converging lines of evidence with taste reactivity, these results showing a significant reduction in cluster size indicate that the palatability of the associated taste CS is diminished following contingent administration of LiCl, the gold standard laboratory agent used to induce toxicosis and CTAs.

## **Experiment 2**

Having demonstrated in Experiment 1 that our procedures can detect a LiCl-induced reduction in the palatability of the associated saccharin CS, Experiment 2 used the same procedure to determine whether a similar effect on saccharin palatability could be obtained using our standard 15 mg/kg dose of morphine as the US (e.g., Lin et al., 2011; Lin, Roman & Reilly, 2009). Using 2, 8, 20, or 80 mg/kg as the US, Parker (1991) reported that, even after five sucrose-morphine conditioning trials, no dose of morphine was effective at producing aversive taste reactivity responses to the associated CS.

## **Method**

**Subjects, apparatus and procedure.** Twenty-four experimentally naïve male Sprague-Dawley rats were obtained and housed as described above. The apparatus employed in Experiment 1 was also used in Experiment 2. The procedure was identical to that described in Experiment 1, except that morphine sulfate (Baxter Healthcare Corporation, Deerfield, IL; 15 mg/ml/kg) served as the US and was injected 5 min after the CS terminated.

## Results and Discussion

Table 1 summarizes the water data collected 24 h prior to the first conditioning trial. Statistical analysis found no significant group differences for water intake ( $p > .20$ ), total licks ( $p > .05$ ), cluster size ( $t < 1$ ), or lick efficiency ( $t < 1$ ).

--- INSERT FIGURE 2 ABOUT HERE ---

As suggested by inspection of Figure 2A, saccharin intake was similar on Trial 1 for both groups but intake in Group Unpaired ( $n = 8$ ) subsequently increased whereas intake in Group Paired ( $n = 16$ ) declined over trials. Analysis found significant main effects of Group,  $F(1, 22) = 19.63$ ,  $p < .05$ , and of Trial,  $F(2, 44) = 6.84$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 44) = 26.02$ ,  $p < .05$ . Follow up comparisons of the interaction confirmed that there was no between groups difference in intake on Trial 1 ( $p < .05$ ). From this common starting point, Group Unpaired increased their intake on Trial 2 ( $p < .05$ ) and stabilized intake from Trial 2 to 3 ( $p > .05$ ). Group Paired, on the other hand, significantly decreased intake after each trial successive trial ( $ps < .05$ ). The pattern of performance seen with total licks per session (Figure 2b) was virtually identical to that for amount of saccharin consumed. ANOVA found significant main effects of Group,  $F(1, 22) = 15.16$ ,  $p < .05$ , and of Trial,  $F(2, 44) = 6.88$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 44) = 34.36$ ,  $p < .05$ . Post hoc analysis of the interaction revealed a pattern of licking identical to that described above for intake. With regard to cluster size (Figure 2C), the repeated measures ANOVA found a significant main effect of Group,  $F(2, 44) = 14.12$ ,  $p < .05$ , as well as a significant Group x Trial interaction,  $F(2, 44) = 4.67$ ,  $p < .05$ ; the main effect of Trial was not significant ( $p > .06$ ). Post hoc analysis of the interaction revealed no between group differences for cluster

size on Trial 1 ( $p < .05$ ). However, Group Unpaired significantly increased cluster size on Trial 2 ( $p < .05$ ) but not from Trial 2 to Trial 3 ( $p > .05$ ). Group Paired showed the opposite pattern such that there was no change in cluster size from Trial 1 to 2 ( $p > .05$ ) and a significant decrease from Trial 2 to Trial 3 ( $p < .05$ ). Lick efficiency data are summarized in Figure 2D. Although there was a large numerical difference between the Paired and Unpaired Groups on Trial 3, once again this measure was prone to large fluctuations in variance such that both the main effect of Group,  $F(1, 22) = 3.93$ ,  $p < .06$ , and the Group x Trial interaction,  $F(2, 44) = 3.01$ ,  $p < .06$ , narrowly failed to achieve significance.

The 15-mg/kg morphine US not only produced a significant decrease in amount consumed and total licks, it also supported a significant reduction in clusters size. This finding of a morphine-induced reduction in taste palatability stands in marked contrast to the null results found using taste reactivity methodology even when extremely high doses of morphine have been used.

### Experiment 3

As reported in the companion article (Lin et al., 2012), a 1 mg/kg dose of d-amphetamine sulfate supported a significant reduction in CS intake, total licks, cluster size, and lick efficiency. Using the same amphetamine US, Dwyer, Boakes and Hayward (2008) reported that although amphetamine reduced CS intake there was no significant reduction in cluster size. One notable difference between these two sets of experiments that may account for the different outcomes concerns the CSs employed. That is, Dwyer et al. used saccharin whereas Lin et al. used quinine, sodium chloride

and aqueous orange odor as CSs. Accordingly, Experiment 3 was conducted to test whether saccharin is immune to amphetamine-induced changes in taste palatability.

## Method

**Subjects, apparatus and procedure.** Sixteen naïve male Sprague-Dawley rats were obtained and housed in Experiment 1. The apparatus and procedures were identical to those described for Experiment 1, except amphetamine sulfate (1 mg/ml/kg; Sigma Aldrich, St. Louis, MO) served as the US and was injected 5 min after the CS.

## Results and Discussion

Analysis conducted on the data from the final baseline trial before conditioning began (see Table 1) found no differences between Unpaired and Paired groups for the amount of water consumed, total licks, cluster size, or lick efficiency (all  $t < 1$ ).

--- INSERT FIGURE 3 ABOUT HERE ---

Figure 3 depicts the saccharin consumption results for Group Unpaired ( $n = 8$ ) and Group Paired ( $n = 8$ ). Saccharin intake patterns (Figure 3A) reveal that Group Paired learned to suppress intake, while Group Unpaired increased intake. A repeated measures ANOVA was used to analyze saccharin intake, and revealed a significant main effect of Group,  $F(1, 14) = 32.40$ ,  $p < .05$ , a significant main effect of Trial,  $F(2, 28) = 6.34$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 28) = 149.54$ ,  $p < .05$ . Follow up statistics of the interaction revealed a significant between groups difference on Trial 1 with Group Unpaired starting significantly lower than Group Paired ( $p < .05$ ). On Trial 2 Group Unpaired significantly increased intake from Trial 1 while Group Paired significantly decreased intake resulting in a significant between groups difference

opposite to that seen on Trial 1 ( $ps < .05$ ). On Trial 3 Group Unpaired showed no change from Trial 2, while Group Paired once again significantly decreased intake from Trial 2 levels ( $ps < .05$ ). Inspection of the data for total licks (Figure 3B) indicates a pattern very similar to that of intake. An ANOVA was conducted to analyze total licks for saccharin revealing a significant main effect of Group,  $F(1, 14) = 22.80$ ,  $p < .05$ , a significant main effect of Trial,  $F(2, 28) = 6.48$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 28) = 157.46$ ,  $p < .05$ . Follow up statistics of the interaction reveal a pattern of results identical to those describe above with regards to intake ( $ps < .05$ ). A mixed ANOVA was conducted to analyze cluster size (Figure 3C) and revealed a significant main effect of Group,  $F(1, 14) = 6.47$ ,  $p < .05$ , and a significant Group x Trial interaction,  $F(2, 28) = 17.22$ ,  $p < .05$ , there was not a main effect of Trial ( $p > .15$ ). Post hoc comparisons of the Group x Trial interaction revealed a significant between groups difference on Trial 1 such that animals in Group Unpaired had significantly smaller cluster sizes than Group Paired. On Trial 2 this pattern was completely reversed such that animals in Group Unpaired significantly increased intake on Trial 2 relative to Trial 1 and were also significantly higher than their Group Paired on Trial 2 who had significantly decreased in cluster size on Trial 2 relative to their Trial 1 performance ( $ps < .05$ ). Both groups failed to show any change in cluster size between Trial 2 and Trial 3 with Group Unpaired remaining significantly higher than Group Paired ( $ps < .05$ ). The ANOVA conducted on the lick efficiency results summarized in Figure 3D revealed significant main effects of Group,  $F(1, 14) = 9.69$ ,  $p < .05$ , and Trial,  $F(2, 28) = 5.95$ ,  $p < .05$ , as well as a significant Group x Trial interaction,  $F(2, 28) = 7.47$ ,  $p < .05$ . Post hoc analyses confirmed what is evident from inspection of the figure – lick efficiency was

significantly lower in Group Paired than Group Unpaired on Trial 3. These results indicate that as the CS becomes more aversive and less palatable over trials, lick volume declines until the rat stops licking altogether when the CS is too distasteful.

The rats were counterbalanced into groups based on their water intake scores on the day before the first conditioning trial. Because there were no significant group differences in terms of water consumed, total licks, cluster size or lick efficiency, it was surprising that group differences on each measure were found on the first conditioning trial. Since these data were obtained during the first exposure to the saccharin CS, and prior to the injection on that trial, it is not clear why these group differences occurred. However, it is equally clear that the different starting levels for each group on Trial 1 do not compromise interpretation of the results that are consistent with those reported in Experiment 2 and by Lin et al. (2012). Indeed, the high scores on Trial 1 for Group Paired increased the range over which drug-induced reductions of the dependent measures could be evaluated. And, unlike the experiment reported by Dwyer et al. (2008), we found that cluster size was significantly reduced by contingent injections of amphetamine. As discussed in the companion paper (Lin et al.), any one of a number of procedural differences might account for the null effect obtained by Dwyer et al. However, we note that for Group Paired in the latter study cluster size for 0.1% saccharin on Trial 1 (the conditioning trial) was about 32 whereas cluster size for water on the preceding day was 38.6. Compared to the scores obtained from Group Paired in our Experiment 3 (51.5 for 0.15% saccharin and 97.4 for water), it might be that 0.1% saccharin is treated as more familiar than 0.15% saccharin and that taste familiarity differentially influences the rate at which amphetamine reduces palatability relative to



amount consumed and total licks, which were significantly lower on the test trial after the single conditioning trial in the Dwyer et al. experiment. Whatever the reason for the null effect reported by Dwyer et al., the results obtained in Experiment 2 and Experiment 3 as well as by Lin et al. (2012) show that contingent administration of a drug of abuse (morphine and amphetamine) significantly reduces the cluster size of the associated CS. We conclude that drugs of abuse reduce palatability like the illness-inducing agent LiCl and, as such, that they do so through the operation of the same CTA mechanism.

### **Experiment 4**

In addition to illness-inducing agents and drugs of abuse, intake of a saccharin CS can be influenced by consumption of a highly preferred sucrose solution (e.g., 1.0 M). This effect, termed anticipatory negative contrast (ANC), occurs when food deprived rats are given sequential access to saccharin and sucrose as a CS-US pairing once per day (Flaherty & Checke, 1982; Flaherty, Turovsky & Krauss, 1994; Lucas, Gawley & Timberlake, 1988; for a review see Flaherty, 1996). That saccharin intake can be influenced by a sucrose US prompts questions about the nature of the underlying mechanism and whether, like LiCl and drugs of abuse, the conditioned change in CS intake by sucrose is accompanied by a reduction in the palatability of the taste CS. Experiment 4 was designed to address these issues. In the standard ANC procedure, the saccharin CS and sucrose US are each available for 3 min. Such brief durations severely constrain the amounts that can be consumed of each solution. For example, in Flaherty and Check (1982; Experiment 1) total licks for the saccharin CS in the experimental (saccharin-sucrose) group was around 100 licks during the first 7 trials.

Such low intake compromises detection of reductions in cluster size. In an effort to elevate baseline intake, we modified the ANC procedure based on preliminary work. Thus, in Experiment 4, rats were food and water deprived, the CS and US were each 12 min in duration, and the US was 1.5 M sucrose. In terms of design, the rats were assigned to either a control group in which both bottles contained 0.15% saccharin (low reward-low reward; LL) or an experimental group (low reward-high reward; LH) in which bottle 1 contained 0.15% saccharin and bottle 2 contained 1.5 M sucrose.

## **Method**

**Subjects.** Sixteen naïve male Sprague-Dawley rats were obtained and housed as described above.

**Apparatus.** The apparatus used in Experiment 4 was that used in the earlier experiments, except that each chamber was equipped with two retractable stimulus bottles. The access holes for the spout tips were 16.6 cm apart (center-to-center) on the right side wall.

**Procedure.** For the first 5 days in the laboratory, the rats were handled for 5 min each day after they were weighed. All rats were then placed on a food and water deprivation schedule consisting of 24-min water access in the home cage each morning, followed 5 hrs later by 60-min access to food and water also in the home cage. Standard lab chow was available in stainless steel hoppers; all food and water was removed from the cage at the end of the afternoon access period. Animals were maintained at a minimum of 85% free-feeding body weight. Once water intake and body weights stabilized (~10 days), morning water access was shifted to the drinking

chambers where water was available for 12 min from bottle 1 (left bottle) and, following a 0-s inter-stimulus interval, for 12 min from bottle 2 (right bottle). Conditioning trials began when intake and licking behaviors were stable in the drinking chambers (about 4 days). The animals were counterbalanced into a control group (LL;  $n = 8$ ) and an experimental group (LH;  $n = 8$ ) according to performance on the final water intake trial. To prevent cross-contamination with sucrose, each group was run separately in a single squad starting with the LL rats. For each group, one trial was scheduled at the same time each day for a total of 12 trials. One animal was dropped from the LL group because of unstable levels of performance in the drinking chamber reducing the number of rats in that group to 7.

## Results and Discussion

--- INSERT FIGURE 4 ABOUT HERE ---

Figure 4 depicts the CS-directed performance of the LL and LH groups on the three dependent measures over the six 2-trial blocks of the experiment. Inspection of Figure 4A suggests that Group LL recovered from the initial neophobia reaction to the novel saccharin CS and gradually increased intake across blocks, while Group LH, which started at the same level as Group LL, maintained and only slightly increased intake over blocks. A mixed ANOVA confirmed these impressions of saccharin CS intake. Thus, there was a significant main effect of Group,  $F(1, 13) = 17.90$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 23.86$ ,  $p < .05$ , and a significant Group  $\times$  Block interaction,  $F(5, 65) = 6.24$ ,  $p < .05$ . Post hoc analysis of the interaction showed no between group difference on Block 1 ( $p > .05$ ) followed by a significant increase in

the Group LL on Block 2 resulting in a significant between group difference on Block 2 which remained significant across all subsequent blocks ( $ps < .05$ ). Examination of total licks for the saccharin CS (Figure 4B) revealed a pattern similar to that of amount consumed. The ANOVA analyzing total licks revealed a significant main effect of Group,  $F(1, 13) = 17.69$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 16.75$ ,  $p < .05$ , and a significant Group x Block interaction,  $F(5, 65) = 5.58$ ,  $p < .05$ . Post hoc analysis of the interaction revealed a pattern of results identical to that described above for intake ( $ps < .05$ ). Inspection of Figure 4C suggests that cluster size gradually increased in Group LL over blocks, an effect that was notably absent in Group LH. The overall ANOVA on CS cluster size data revealed a significant main effect of Group,  $F(1, 13) = 7.69$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 9.94$ ,  $p < .05$ , and a significant Group x Block interaction,  $F(5, 65) = 2.47$ ,  $p < .05$ . Follow up statistics found no between groups difference on Block 1 followed by a significant increase in CS cluster size for the LL group on Block 2 creating a significant between groups difference on Blocks 2, 3, and 6 ( $ps < .05$ ). This pattern of results indicates that pairing saccharin with sucrose limits the increase in cluster size to the associated CS that would otherwise occur, as seen in Group LL. Lick efficiency results are displayed in Figure 4D. Statistical analysis revealed a significant main effect of Group,  $F(1, 13) = 9.48$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 9.24$ ,  $p < .05$ , and a significant Group x Block interaction,  $F(5, 65) = 7.05$ ,  $p < .05$ . Follow-up statistics found no across-block changes in the lick efficiency of Group LL ( $ps > .05$ ). For Group LH, however, post hoc analysis revealed a significant decrease in lick efficiency on Block 1 relative to Blocks 2 - 4 ( $ps < .05$ ) and a significant increase in lick efficiency on Blocks 5 and 6 relative to Block 1 (or

Blocks 2 – 4;  $ps < .05$ ). Although it is clear that the performance of Group LL remained stable across successive Blocks, we have no explanation for the pattern of lick efficiency changes manifest by Group LH.

--- INSERT FIGURE 5 ABOUT HERE ---

Figure 5 shows the US-directed performance for saccharin in Group LL and for sucrose in Group LH over the six 2-trial blocks of the experiment. As shown in Figure 5A, intake of saccharin in Group LL remained low across blocks (less than 3 g) whereas Group LH showed an initial neophobic reaction to the novel sucrose solution that quickly habituated across blocks to stabilize at about 12 g. The ANOVA conducted on US intake found a significant main effect of Group,  $F(1, 13) = 73.93$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 14.25$ ,  $p < .05$ , and a significant Group x Block interaction,  $F(5, 65) = 7.15$ ,  $p < .05$ . Post hoc analysis revealed that the LH group consumed significantly more sucrose than Group LL drank of saccharin during Block 1 that and this between groups difference significantly increased on Block 2 ( $p < .05$ ), and this between groups difference remained stable Blocks 3 - 6. An identical pattern of significance was found for total licks (see Figure 5B). Thus, the initial ANOVA found a significant main effect of Group,  $F(1, 13) = 74.10$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 5.65$ ,  $p < .05$ , as well as a significant Group x Block interaction,  $F(5, 65) = 2.50$ ,  $p < .05$ , and the post hoc analysis confirmed that the between group differences in total licks were identical to those described above for amount consumed ( $ps < .05$ ). Inspection of the data in Figure 5C shows that cluster size for Group LL remained low and constant across blocks whereas for Group LH cluster size started low

and incremented across blocks such that cluster size quadrupled from Block 1 to Block 6. The ANOVA conducted on the US cluster size data revealed a significant main effect of Group,  $F(1, 13) = 16.96$ ,  $p < .05$ , a significant main effect of Block,  $F(5, 65) = 8.25$ ,  $p < .05$ , and a significant Group x Block interaction,  $F(5, 65) = 5.40$ ,  $p < .05$ . Post hoc analysis found no between group difference on Block 1 ( $p > .05$ ) followed by a significant increase in Group LH on block 2 resulting in a significant between groups difference on block 2, which was also significant on blocks 4, 5, and 6 ( $ps < .05$ ). The overall pattern of results suggest, as expected, that sucrose is a more preferred solution than saccharin.

The results of Experiment 4 are important for a number of reasons. First, they confirm that our procedural modifications supported the acquisition of an ANC effect which was substantially larger than that obtained with the standard parameters. Second, these modifications successfully elevated response levels such that a reduction in performance could not be obscured by a floor effect. Third, there was no evidence that the sucrose US caused, relative to Block 1, a significant reduction in the palatability of the associated saccharin CS or, indeed, amount consumed or total licks. The effect of the sucrose US is best characterized as maintaining the initial values of the three dependent measures that define CS-directed behavior in this ANC task. Although, as is evident from inspection of Figure 4, there was a tendency for the values of three dependent measures (intake, total licks and clusters) to move upwards over repeated CS-US pairings towards the levels of performance of the LL control group. Fourth, these results clearly demonstrate that the performance directed at the saccharin CS that was paired with the sucrose US was not similar to the CS-directed performance when LiCl,

morphine, or amphetamine was used. Overall, the results from Experiment 4 suggest that the sucrose US is influencing learning in a manner that is qualitatively different from illness-inducing agents and drugs of abuse.

With regard to the performance of the LL control group, we see the habituation of taste neophobia was manifest not only as an increase in amount consumed and total licks but also an increase in cluster size (i.e., an increase in palatability). A similar pattern of recovery from neophobia is evident in the Unpaired control animals in Experiments 1 - 3, which were, it will be recalled, also exposed to noncontingent drug injections (i.e., LiCl, morphine, or amphetamine, respectively) constituting a potential confound. No such confound occurred in Group LL of Experiment 4. Although a single non-confounded demonstration is hardly conclusive, the similar patterns of performance in the control animals of all four experiments encourages a more systematic examination of palatability during the initial occurrence of, and the recovery from, taste neophobia, an investigation that is currently underway in our laboratory.

### **General Discussion**

In a series of four experiments we paired a standard taste CS (0.15% saccharin) with four USs (LiCl-induced illness, morphine, amphetamine, or sucrose), while recording the temporal microstructure of lick patterns during short periods (15 or 12 min) of voluntary drinking. Intake, total licks, mean cluster size (a measure of palatability) and lick efficiency were monitored during each experiment. Experiment 1 replicated previous studies (e.g., Baird et al., 2005; Dwyer, 2009; Dwyer et al., 2008; Kent et al., 2002) showing that LiCl conditions a significant reduction in the amount of CS consumed as

well as a significant reduction in cluster size. Thus, as a consequence of CTA learning, the CS becomes disliked or distasteful. In other words, LiCl-induced illness devalues the taste CS such that it is unpalatable and therefore avoided or rejected during future encounters. CTA is not a finely tuned mechanism. To the contrary, as might be expected for a mechanism that evolved to protect against ingested poisons, the mechanism would have maximum adaptive value if it were very broadly tuned to associate a new taste (or flavor) with any subsequent perceived internal malaise. Presumably, different poisons or toxins have different malaise properties such that the more broadly tuned the CTA mechanism the greater its survival value. This type of mechanism would, of course, be prone to false positives (i.e., the development of CTAs to a food that did not, in fact, cause the subsequent illness). But, when the threat is potentially a fatal poisoning the occurrence of false positives is a small price to pay. Perhaps the most obvious examples of false positives are, unfortunately, the CTAs that cancer patients develop despite full awareness that the chemotherapy drugs, not the previously consumed food, caused their internal malaise (for a recent review see Scalera & Bavieri, 2009).

When saccharin was paired with either morphine (Experiment 2) or amphetamine (Experiment 3), a significant decrease in saccharin intake and a concomitant reduction in cluster size were obtained. This decrease in CS cluster size is comparable to the reduction in cluster size that was observed with the illness-inducing agent LiCl in Experiment 1. Accordingly, we conclude that morphine and amphetamine, and perhaps drugs of abuse in general, cause a reduction in the palatability of the associated CS. Thus, we are inclined to the view that drugs of abuse induce CTAs. These conclusions



are very different to those derived from research that employed taste reactivity methodology which has consistently failed to find evidence of a shift in palatability to a taste CS that was paired with a drug of abuse US. In hindsight, close examination of this literature shows that in some instances (e.g., Parker, 1991, 1993, 1996; Davies & Wellman, 1990) there are indications of trends towards a shift in palatability as reflected by a decrease in ingestive orofacial responses and an increase in aversive responses. That such trends in performance were not sufficiently large to be interpreted as a shift in taste palatability indicates that taste reactivity methodology may be less sensitive than microstructural analysis of lick patterns in the detection of conditioned shifts in taste palatability.

That morphine and amphetamine, and other psychoactive drugs that have very different pharmacological effect profiles, can induce CTAs begs the question: what is the common effect underlying the taste aversions conditioned by drugs of abuse? As noted above, we view CTA as a broadly tuned defense mechanism that protects against the ingestion of poisonous foods but which is liable to false positives; it is better, in other words, to be over cautious than not cautious enough in such situations. From this perspective, any agent or event that alters the normal internal state of the body in a way that can be construed as similar to that of a poison or toxin might, correctly or incorrectly, be perceived as a poison or toxin and become associated with a novel tasting food that was recently consumed. It is possible, then, that CTAs induced by drug of abuses, drugs that otherwise are rewarding, are another type of false positive. That is, in feeding situations (particularly those involving a novel tasting food), whether the internal state produced by the drug is accurately or mistakenly perceived as evidence of

poison, that state will usurp the CTA defense mechanism. In this particular situation, it matters little that the drug might, in reality, not be poisonous or toxic because that misattribution of causality has triggered the engagement of a mechanism whose purpose is to devalue the associated taste causing a reduction in palatability that will discourage future consumption of that food. On the other hand, in non-feeding situations, the very same drug of abuse might be self-administered and its rewarding properties support the development of, for example, a conditioned place preference (e.g., Berger, 1972; Reicher & Holman, 1977; Wise, Yokel & DeWit, 1976). Furthermore, these two very different behavioral outcomes, a taste aversion and a place preference, can be produced concurrently by the same drug injection (Verendeev & Riley, 2011; Wang, Huang, & Hsiao, 2010).

In the ANC task of Experiment 4, the sucrose US had a very different influence on learning relative to the US effects found in Experiments 1-3. That is, unlike LiCl-induced illness, morphine or amphetamine, the sucrose US did not produce significant reductions in CS intake or cluster size relative to performance on block 1. Rather, the sucrose US seemed to attenuate the increase in saccharin consumption and cluster size that would otherwise occur, as shown in the performance of the LL control group. Indeed, as inspection of Figure 4 shows, there is some suggestion that three CS performance scores (intake, total licks and cluster size) of the LH group were increasing across trials. We are left to speculate whether, given more saccharin-sucrose pairings, the performance of the LH group would eventually match that of Group LL. At best, then, the sucrose US may be considered to maintain the palatability of the CS at the same level as that defined by the neophobic reaction to the novel saccharin on first

encounter. There is no evidence whatsoever that the sucrose US influenced CS palatability in the same manner as the USs in Experiments 1-3. Accordingly, we consider that the mechanism responsible for the ANC effect is qualitatively different from the CTA mechanism engaged by LiCl-induced illness, morphine and amphetamine. Moreover, the present results demonstrate that ANC is not due to the devaluation of the saccharin CS by the sucrose US, a conclusion that other investigators have reached based on less direct methods of assessment (e.g., Capaldi & Sheffer, 1992; Flaherty, Coppotelli, Grigson, Mitchell & Flaherty, 1995; Lucas & Timberlake, 1992).

There are, it might be noted, some procedures in which sucrose does influence the palatability of another solution. For example, Dwyer, Lydall and Hayward (2011) recently reported that in a simultaneous contrast task, positive contrast (elevated intake of 32% sucrose when the within-session comparison was 4% sucrose rather than the same 32% sucrose) and negative contrast (reduced intake of 4% sucrose when the within-session comparison was 32% sucrose rather than the same 4% solution) were accompanied by, respectively, a significant increase and a significant decrease in palatability as assessed using cluster size. Furthermore, Grigson, Spector and Norgren, (1993) reported that successive negative contrast (reduced intake of unexpected 0.1 M sucrose relative to intake of an expected 0.1 M sucrose solution) was also evident as a reduction in cluster size (termed bursts by the authors but defined like clusters in the present study) to the unexpected 0.1 M sucrose solution. These findings demonstrate that these two forms of incentive contrast effects produce shifts in palatability (negative contrast effects cause stimulus devaluation, while positive contrast effects enhanced

stimulus valuation). Although simultaneous contrast and successive negative contrast are each transient phenomena and produced by different mechanisms from each other and ANC (see Flaherty, 1996, for a review), they do, of course, highlight the sensitivity of cluster size in tracking changes in palatability. However, as the present investigation confirmed with ANC, there is no suggestion that sucrose can condition a decrease in palatability even in a procedure designed to optimize the detection of any such reductions.

To summarize, we found that morphine and amphetamine each caused a significant reduction in the intake and palatability of the associated saccharin CS, a pattern identical to that found with LiCl-induced illness. This pattern of results, we suggest, supports the conclusion that drugs of abuse induce CTAs. Furthermore, we suggest that exposure to a taste stimulus prior to drug administration gates the drug induced internal state into the category of post-ingestive feedback. This allows the drug state, whether aversive or rewarding, to be interpreted as a sign of poisoning and to become associated with the taste CS leading to the development of an aversion marked by a reduction in taste palatability. In the absence of an internal cue such as taste, the rewarding properties of the drug may be associated with external cues as might occur, for example, in the conditioned place preference task.

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Correspondence concerning this article should be addressed to Jian-You Lin or Steve Reilly, Department of Psychology, 1007 West Harrison Street, University of Illinois at Chicago, Chicago, Illinois 60607. E-mail: [jlin2@uic.edu](mailto:jlin2@uic.edu) or [sreilly@uic.edu](mailto:sreilly@uic.edu)

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Table 1. Water consumption from Experiments 1, 2 and 3 was assessed using four dependent measures: intake, total licks, cluster size, and lick efficiency. Values presented are the means ( $\pm$ SE), from the final water day before the first conditioning trial for the control (Unpaired; saccharin-saline) and experimental (Paired; saccharin-US) groups.

Experiment	Group	Intake (g)	Total licks	Cluster size	Lick efficiency
1	Unpaired	11.49 (0.73)	2338.13 (199.22)	83.00 (15.75)	203.36 (11.41)
	Paired	11.64 (1.10)	2227.38 (225.41)	95.16 (17.09)	196.44 (18.67)
2	Unpaired	14.35 (.94)	2521.25 (133.21)	94.00 (17.23)	176.94 (4.00)
	Paired	16.07 (0.80)	2840.75 (102.71)	120.99 (22.32)	179.71 (5.98)
3	Unpaired	16.21 (0.80)	2621.00 (91.45)	105.68 (17.58)	164.41 (10.32)
	Paired	15.15 (0.83)	2571.38 (92.06)	97.43 (16.70)	171.19 (5.13)



### Figure Captions

Figure 1. Experiment 1. Mean ( $\pm$ SE) conditioned stimulus-directed performance for lithium-based taste aversion in Group Unpaired and Group Paired across the three trial of the experiment. Panel A, intake (g); panel B, total licks; panel C, cluster size; panel D, lick efficiency.

Figure 2. Experiment 2. Mean ( $\pm$ SE) conditioned stimulus-directed performance for morphine-induced taste aversion in Groups Unpaired and Paired during the three trial of the experiment. Panel A, intake (g); panel B, total licks; panel C, cluster size; panel D, lick efficiency.

Figure 3. Experiment 3. Mean ( $\pm$ SE) conditioned stimulus-directed performance for amphetamine-induced taste aversion in Group Unpaired and Group Paired across the three trial of the experiment. Panel A, intake (g); panel B, total licks; panel C, cluster size; panel D, lick efficiency.

Figure 4. Experiment 4: Mean ( $\pm$ SE) conditioned stimulus-directed performance during anticipatory negative contrast for the control LL group and the experimental LH group. Panel A, intake (g); panel B, total licks; panel C, cluster size; panel D, lick efficiency.

Figure 5. Experiment 4: Mean ( $\pm$ SE) unconditioned stimulus-directed performance during anticipatory negative contrast for the control LL group and the experimental LH

group. Panel A, intake (g); panel B, total licks; panel C, cluster size; The unconditioned stimulus was 0.15% saccharin for Group LL and 1.5 M sucrose for Group LH.

Figure 1

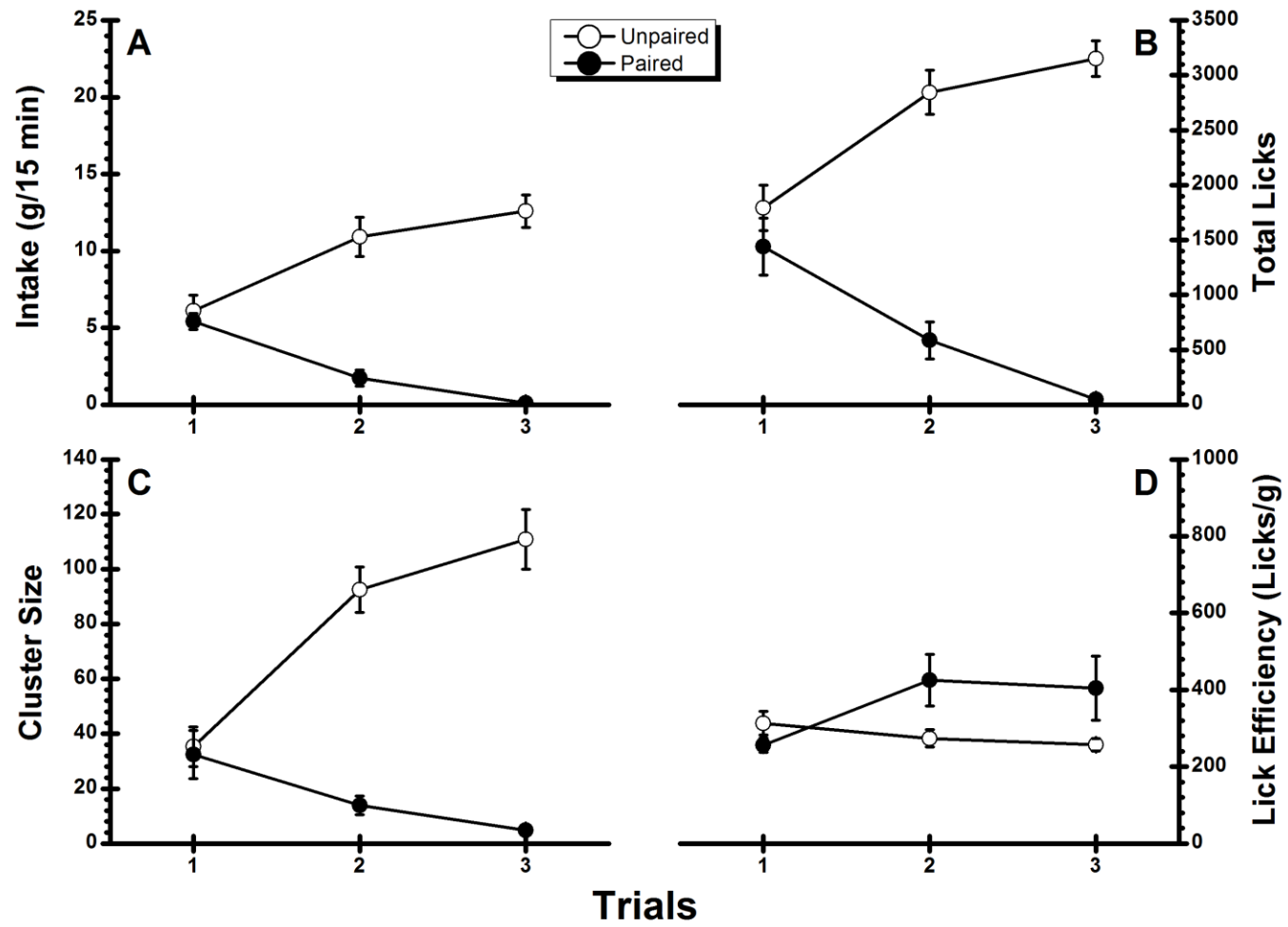


Figure 2

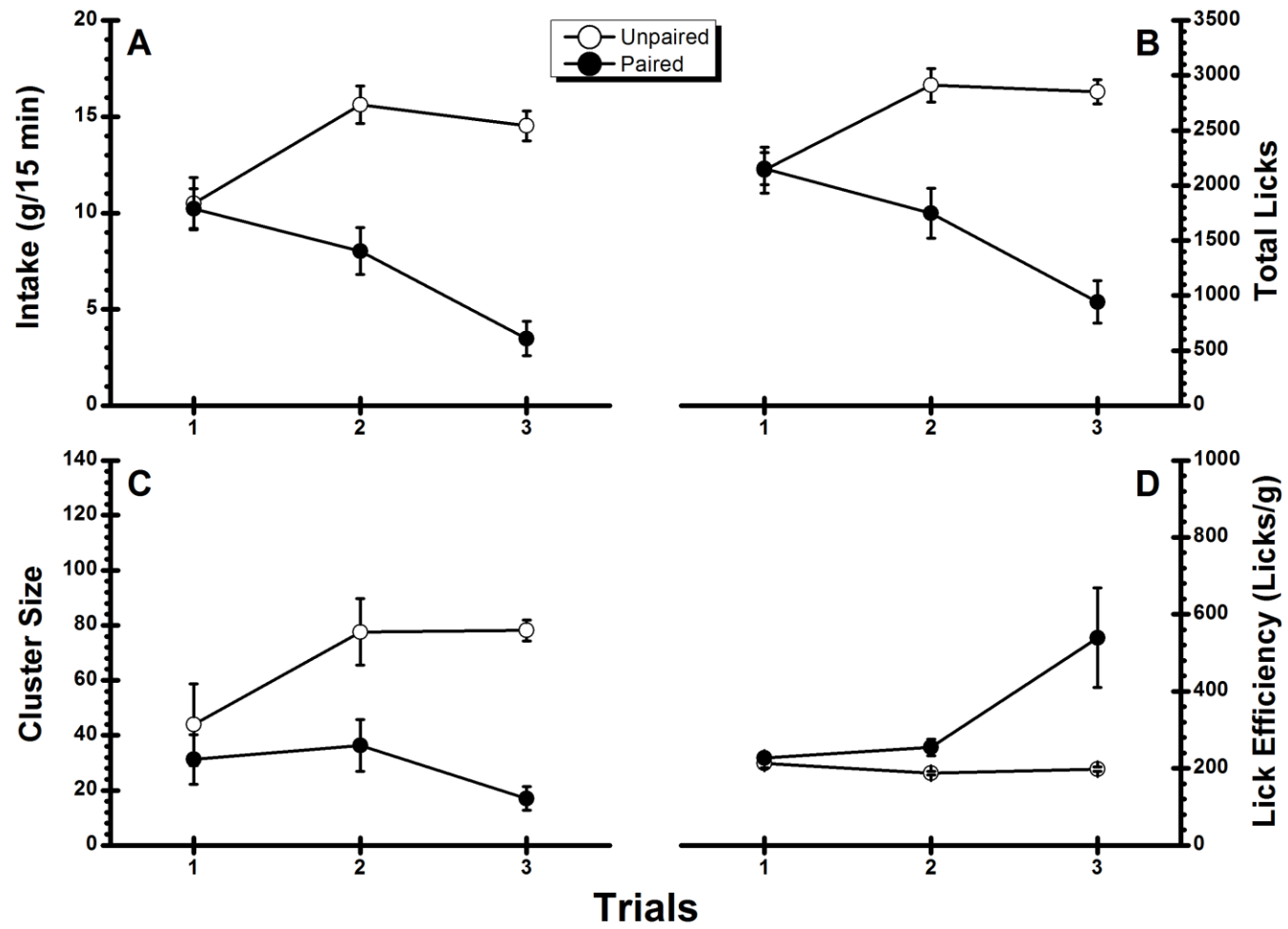
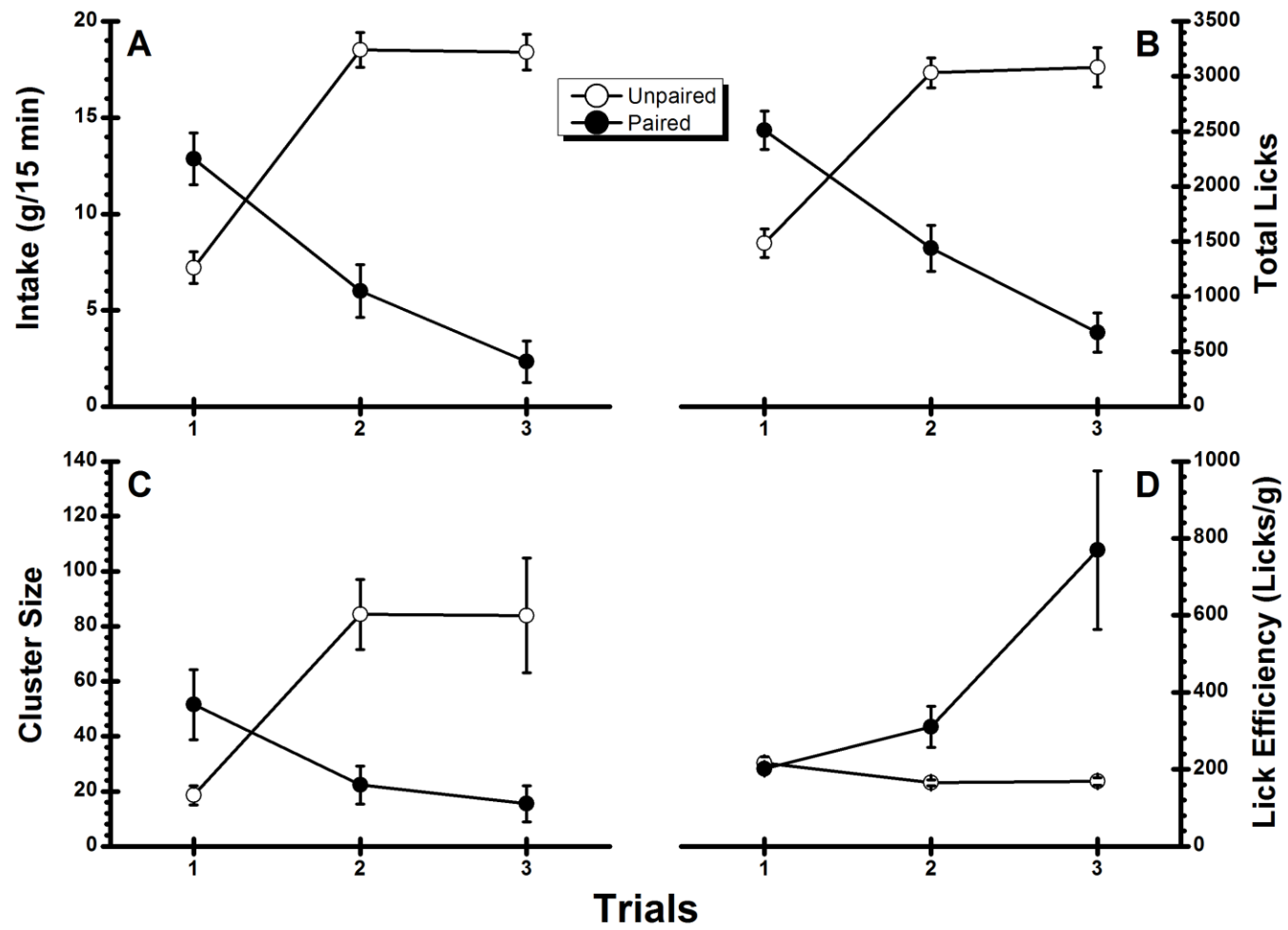


Figure 3



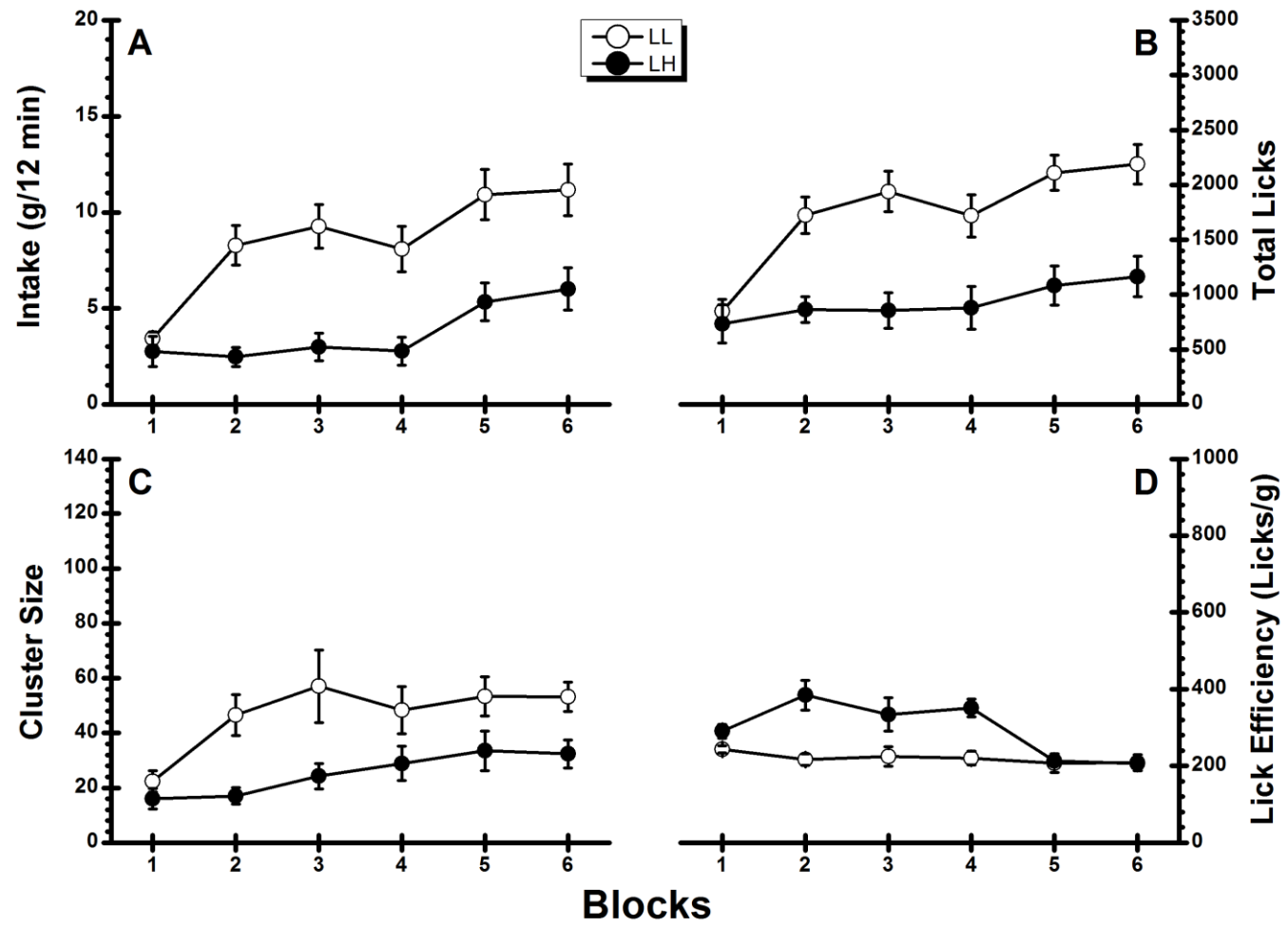


Figure 4

Figure 5

