Contextual Control of Aversive and Avoidance Responding in a Flavor Learning Paradigm in Rats

by

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Abstract

Contextual control over aversive responding was examined in two experiments where rats were trained on a context discrimination task; a saccharin solution was paired with lithium chloride (LiCl) in one context but paired with saline in a different context. In Experiment 1, rats drank significantly less in the danger context than in the safe context, and showed aversive orofacial responses in the danger context. Contextual control transferred to a novel solution. Ten extinction trials, where rats were exposed to the danger context alone or with tap water in the danger context, produced a strong reduction in contextual control over aversive responding and a weak reduction in avoidance of the trained flavor. In Experiment 2, additional groups were added to the group described above. One group received tap water rather than saccharin solution during discrimination training, while another group did not receive fluid in the training context. Again, contextual control transferred to a novel flavor and all groups showed aversive responding in the danger context and avoidance of the danger context on a place choice test. Post-extinction tests revealed that context avoidance was weakest in the group trained with saccharin solution, even though that group showed the slowest extinction of contextual control over consumption. Additionally, differential gaping was slower to appear and faster to extinguish than chin rubbing in all groups, suggesting that these responses have different underlying mechanisms. Associative mechanisms underlying contextual control over aversive and avoidance responses are discussed.

Keywords: gaping, chin rubbing, taste aversion, taste avoidance, overshadowing, contextual cues

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Consuming foods that are followed by positive consequences and avoiding foods that are followed by negative consequences is an important learning skill seen in many species. Early studies that paired a novel flavor (conditioned stimulus; CS) with an illness-inducing agent (unconditioned stimulus; US) showed that animals decreased consumption of the flavor when it was offered in later trials (Garcia, Hankin, & Rusiniak, 1974; Revusky & Garcia, 1970). Animals that lack the ability to vomit, such as rats, are especially good at avoiding foods previously paired with illness since they cannot remove a poison quickly after it has entered their system (Parker, 2003).

Later studies revealed that this flavor avoidance could be put under discriminative control by contextual cues (Bonardi, Honey, & Hall, 1990; Murphy & Skinner, 2005; Nakajima, Kobayashi, & Imada, 1995; Puente, Cannon, Best, & Carrell, 1988; Skinner, Martin, Pridgar, & van der Kooy, 1994). For example, if illness is induced following consumption of a novel flavor in one context, but not in a second context, animals will learn to ingest the flavor only in the unpaired context. This discriminative control, evidenced by the differential consumption of the flavored solution, has been demonstrated with contexts defined by several modalities, including drug states (Martin, Riccio, & Riley, 1988; Skinner et al., 1994), brightness (Brown, Penney, Skinner, & Martin, 2011; Limebeer et al., 2008), and a combination of odor, brightness, and texture (Murphy & Skinner, 2005; Skinner et al., 1994).

Although contextual control over flavor avoidance has been well documented (Nakajima, Kobayashi, & Imada, 1995; Puente, Cannon, Best, & Carrell, 1988; Skinner et al., 1994; Skinner et al., 2000), the exact mechanism underlying this contextual control has been a topic of debate. The three most prominent explanations in the literature are summation, taste-potentiated context aversion, and occasion setting. According to the summation view, the context enters into a Pavlovian association with the emetic agent (such as lithium chloride; LiCl) and the acquired aversive properties of the context sum with the aversive properties of the flavor (Boakes, Westbrook, Elliott, & Swinbourne, 1997; Bouton, 1993; Loy, Alverez, Rey, & Lopez, 1993; Skinner et al., 1994). The summed associative strength of the context and flavor produces more suppression in the paired context than in the unpaired (safe) context.

The second explanation for contextual control over flavor avoidance also depends on the formation of a context-illness association but in this case the taste potentiates conditioning to the context. In most forms of classical conditioning, a strong cue will overshadow a weak cue when both are paired with a US (Rescorla & Holland, 1982). For example, if a loud noise and a weak light are simultaneously paired with food, more conditioned responding will be observed to the stronger noise than to the weaker light. In contrast, presenting a compound consisting of a weak cue and a flavor paired with a US results in better conditioning to the weak cue than if it had been paired with the US directly (Holder & Garcia, 1987; Westbrook & Brookes, 1988). For example, pairing an odour with LiCl results in weak conditioning to the odour cue. Conditioning to the odour cue is potentiated if the odour is presented with a flavor. Loy, Alvarez, Rey, and Lopez

(1993) claimed that discriminative performance during context discrimination training was due to simple context-illness associations that were enhanced by the availability of a flavor. Westbrook and Brookes (1988) also suggested that a flavor-illness association potentiated the acquisition of a context aversion.

The context discrimination procedure is formally similar to an occasion setting task (Holland, 1983; 1989; 1990; 1992; 1995; Holland & Forbes, 1982; Holland & Ross, 1981). In occasion setting, a cue tells the animal when a particular CS will be followed by a US. On some trials, the CS is presented alone and no US is given. On other trials, the occasion setter cue is given prior to the CS-US pairing. Thus, the occasion setter modulates, or facilitates, the CS-US association (Holland, 1989). With respect to contextual control over taste avoidance, it is thought that the context acts as an occasion setter to modulate the taste-illness association (Boakes, Westbrook, Elliott, & Swinbourne, 1997). In other words, the context indicates whether or not a flavor is safe to consume. The occasion setting function of a cue is believed to be independent of the simple association between the cue and the US (Boakes et al., 1997; Rescorla, 1991; Rosas, & Bouton, 1997). This idea is supported by the fact that simple conditioning to the cue can be extinguished leaving its occasion setting abilities intact (Holland, 1989).

All of the studies mentioned above, demonstrating contextual control over flavor avoidance, have examined flavor avoidance as a single concept, with decreased consumption of a flavor interpreted as the result of a single mechanism of flavor learning. However, other research points to a distinction between this taste avoidance and taste aversion (Cross-Mellor, Kavaliers, & Ossenkopp, 2004; Parker, 1995; 2005). Taste

avoidance is commonly defined as a learned tendency of an animal to avoid consuming a flavor, a response often induced by the paired presentation of a distinct flavor and drug-induced nausea (Best, Brown, & Sowell, 1984; Revusky & Garcia, 1970; Symonds, Hall, Lopez, Loy, Ramos, & Rodriguez, 1998). Taste avoidance is best analyzed by measuring how much of the flavored substance is consumed, either as the only available option (Brown et al., 2011) or compared to the consumption of a co-present, untrained flavor (Cantora et al., 2006). In comparison, taste aversion is known as the dislike for a flavor as a result of a learned association between the flavor and illness. Animals display several disgust reactions and behaviors in the presence of an illness-paired flavor, and these are considered indicative of an aversion to the flavor. Parker (2003) suggested that although taste avoidance and taste aversion often occur simultaneously, they develop by two different processes. Although the two are related, processes that are necessary for the production of taste aversion do not always cause taste avoidance. Similarly, when nausea is reduced, taste aversion is disrupted while taste avoidance may be left unaffected.

There are several other pieces of evidence suggesting that taste aversion and taste avoidance involve separate processes. Established taste avoidance has been shown to extinguish more slowly than a co-acquired taste aversion (Cantora et al., 2006), while the reverse has been observed in preweanling rats (Arias et al., 2010). Similarly, an established flavor preference extinguishes more slowly than co-acquired appetitive responses to the flavor (Dwyer et al., 2009). However, the results of both studies involving a negative palatability shift were obtained using cannulae to present animals with a trained flavor, while the results of Dwyer et al. (2009) were obtained by allowing

rats to drink a fluid from a drinking tube. Differences have been found in the extinction of avoidance of a flavored solution depending on the use of cannulae or one- or two-bottle tests, with flavor presentation via intra-oral cannulae during acquisition leading to faster extinction of the flavor avoidance (Foquet, Oberling, & Sandner, 2001; Wolgin & Wade, 1990; Yamamoto, Fresquet, & Sandner, 2002). Regardless of the method of flavor presentation, changes in the volume of a flavored solution consumed take longer to return to preconditioning levels than the frequency of the aversive or appetitive behaviors that develop simultaneously.

Several studies have investigated the roles of separate brain regions in the learning of taste aversion and taste avoidance. Lesioning the basolateral amygdala attenuates the development of taste avoidance without impacting the acquisition of a conditioned taste aversion (Rana & Parker, 2008). Piasecki et al. (2001) also showed that lesions of the dorsal raphe nucleus attenuated flavor avoidance. However, Limebeer, Parker and Fletcher (2008) found that lesioning the dorsal raphe nucleus attenuated aversive responding but left flavor avoidance intact. Despite the lack of a clear behavioral effect in the few lesion studies completed at this time, the fact that several manipulations can attenuate one process without affecting the other suggests that taste aversion and taste avoidance develop via separate mechanisms.

Both taste avoidance and taste aversion can come under the control of contextual cues. Brown et al. (2011) simultaneously assessed flavor avoidance, by measuring fluid consumption, and flavor aversion, by measuring the orofacial responses of rats to the fluid, in a contextual discrimination task. The rats were placed in either a safe or danger

context for 20 min and presented with a saccharin solution through a drinking tube halfway through each trial. Injections of saline were given following safe trials and injections of LiCl were given following danger trials. Over the course of 10 training cycles (safe day-danger day-safe day) the rats avoided the flavor in the danger context relative to the safe context and exhibited aversive behaviors in the danger context. Two of the aversive behaviors measured by Brown et al. were gaping and chin rubbing. Behaviorally similar to the gaping exhibited by shrews prior to vomiting (Parker, 2003), the gaping response in rats, which cannot vomit, is described as a fast and repeated opening and closing of the jaw. Brown et al. (2011) observed gaping on danger trials both before and after the exposure to saccharin. Gaping to the context cue alone suggests an anticipatory nature to gaping that may be comparable to nausea experienced by humans in response to stimuli that were previously experienced with illness. Chin rubbing, in which the rat rubs its chin quickly across a surface, is also a measure of nausea and occurs alongside gaping and other disgust reactions (Brown et al, 2011; Grill & Norgren, 1978; Limebeer & Parker, 2006). Although chin rubbing and gaping often occur together, evidence suggests that they may function somewhat differently (Parker & MacLeod, 1991) with chin rubbing, but not gaping, showing attenuation in response to an antiemetic drug. In support of different functions of gaping and chin rubbing, Brown et al. (2011) showed that while gaping was more frequent in the first half of the trial and decreased thereafter, the chin rubbing response did not appear to vary significantly within a trial.

Brown et al. (2011) showed that contextual control over flavor avoidance and aversion was acquired at the same rate. However, earlier work showed that taste

avoidance was slower to extinguish than taste aversion. It is not known whether extinction of contextual control over these two processes occurs at differential rates, since most studies that have examined contextual control have only measured consumption, and not the orofacial responses indicative of aversion. For example, Murphy and Skinner (2005) trained rats on a conditional discrimination task in which a saccharin solution was paired with LiCl in one context but paired with saline in another context. Rats drank less saccharin solution in the danger context than in the safe context and also avoided the danger context on a place choice test. The authors suggested that contextual control over flavor consumption was due to an occasion setting mechanism and that avoidance of the danger context on the choice test was due to a Pavlovian association between the context and LiCl. After discrimination training, the rats were given extinction trials with the danger context alone or with the danger context and water. Extinction trials with the context and water abolished contextual control over saccharin consumption but not the avoidance of the danger context on the choice test. Extinction trials with the context alone abolished avoidance of the danger context but not contextual control over saccharin consumption. Thus, extinction of the flavor avoidance depended on the type of extinction procedure employed. There was no independent measure of flavor aversion in that study. Flavor avoidance in the danger context was also retained when extinction occurred with the trained flavor in a second context compared to when extinction took place with the flavor in the conditioning context (Rosas & Bouton, 1997). These results suggest that exposure to the danger context or flavor alone is insufficient for complete extinction of conditional control over taste avoidance. Research on the extinction of taste aversion, as

measured by disgust reactions rather than consumption, has been largely focused on differences between aversion and avoidance extinction within one extinction condition, as seen in the results summarized above (Arias et al., 2010; Cantora et al., 2006; Dwyer et al., 2009), while no comparisons have been made between the extinction of avoidance and aversion responding within a contextual discrimination paradigm.

The overall purpose of the present study was to further examine contextual control over flavor aversion and avoidance using the paradigm established by Brown et al. (2011). The three main sub-goals were to first replicate the basic finding of Brown et al. (2011) using more subjects and look more closely at the distribution of gaping and chin rubbing responses over the duration of the trial. The second sub-goal was to determine if contextual control over flavor avoidance and flavor aversion extinguishes at a similar rate. The final sub-goal was to begin to examine the associative structure of contextual control over flavor aversion, as has been previously done for contexutal control over flavor avoidance.

Experiment 1

Using a contextual discrimination task, Brown et al. (2011) showed that gaping in the danger context was much more frequent in the first 10 min period (before the fluid was introduced) than in the second 10 min period (during fluid presentation). In comparison, chin rubbing did not appear to vary significantly within a trial, with responding occurring at a relatively high level throughout each danger trial. As Brown et al. (2011) only tested eight animals in their experiment the inconsistency seen in gaping may be a result of high individual variability combined with a small subject pool. The

changes in gaping within a trial may have been due to fatigue, within-trial extinction, or a bigger context aversion than taste aversion. However, it is not known why this within-trial decrease occurred with gaping but not with chin rubbing, as both are thought to be measures of taste aversion. The first aim of Experiment 1 was to replicate the basic finding of Brown et al. (2011) using more subjects and to look more closely at the distribution of gaping and chin rubbing responses over the duration of the trial. Sixteen rats were trained on the context discrimination task for 10 cycles. The distribution of gaping and chin rubbing responses was assessed at 1-min intervals during safe and danger trials. Upon completion of the initial acquisition phase, the rats were given two test trials: in one test, the rats were exposed to the danger context for 20 min in the absence of the training flavor and in the second test the flavor was presented for the entire 20 min trial. The purpose of the tests was to determine the contribution of flavor and context aversion to the aversive responses observed during training.

Upon completion of the tests mentioned above, the rats were given a transfer test with a novel flavored solution in the two training contexts. While Skinner et al. (1994) argued that transfer of contextual control over fluid consumption was indicative of contextual modulation of drinking, others have suggested that such transfer effects are indicative of a context aversion (Iguchi, Fukumoto, Sawa, & Ishii, 2014; Loy et al.,1993). However, these previous studies did not examine aversive orofacial responses. Finally, the rats were given extinction trials with the danger context alone or the danger context with water available for consumption. Aversive responses to the danger context were

assessed during the extinction trials and during the retention test with the training flavor at the end of extinction.

Method

Subjects. Sixteen male Long-Evans rats, obtained from the Charles River Company (St. Constant, Quebec, Canada) and weighing between 152 g and 178 g at the start of training, were used. The rats were individually housed in clear plastic cages (45 x 25 x 21 cm) with metal lids in a temperature controlled (20 ± 2 ºC) colony room and maintained on a 12-hr/12-hr light/dark cycle with lights on at 0800. The rats were allowed continuous access to food in their home cages but were kept on a water deprivation schedule starting one week prior to behavioral training. The water deprivation regime consisted of 60 min access to tap water each day, which occurred approximately three hours following the training session. Behavioral training took place five days a week between 0830 and 1230. Animal care and all procedures used in the experiment were approved by Memorial University‘s Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Apparatus. Six rectangular wooden boxes similar to those utilized by Brown et al. (2011; inner dimensions 25.40 x 15.24 x 38.10 cm), three painted white and three painted black, were used. Two small holes were drilled in each box, one on a long wall and one on a short wall, to allow for the introduction of a drinking tube into the box. There was no bottom to the boxes as they were placed on a clear glass table (85.10 x 85.10 cm and 73.70 cm above the floor) for videotaping from below. Four 60 W lights were placed under the corners of the table and pointed toward the middle of the glass, so

that rats were clearly visible for coding on video recordings. A high-definition digital video camera (Canon HFM300 and Canon HFM400) was used to record selected trials from below the box and glass table. Background music was played during all sessions.

During training, rats were given access to a 0.1% saccharin solution in the boxes. Upon completion of training trials, all rats were given an intra-peritoneal (ip) injection of either saline (0.9% NaCl; 3.0 ml/kg) or lithium chloride (LiCl) (0.47 M; 3.0 ml/kg).

Discrimination training. Rats were trained on a context discrimination task that was divided into safe and danger days. On safe days, the rats were placed in either a black or white box for a 10 min period followed by a 10 min access to the saccharin solution while in the same box (20 min total exposure to the context). Removal of the saccharin solution was followed by an ip injection of saline and the rats were returned to their home cages. On danger days, the rats were placed in the alternate context for 10 min followed by 10 min access to the same saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of LiCl and the rats were returned to their home cages. The wooden boxes and glass table were cleaned following each trial. Half of the rats were placed in the white box on safe days and the black box on danger days and the other half received the opposite contingency. Also, for half the rats the spout of the saccharin bottle was placed into the hole on the short wall on safe days and the long wall on danger days. The other half of the rats received the alternate placement. The amount of saccharin solution consumed (in g) each day was recorded.

The acquisition phase was divided into ten 3-day cycles consisting of a danger (D) day preceded and followed by a safe (S) day (S-D-S). Sometimes additional safe days

were required for saccharin consumption to increase before the next cycle was given, such that the actual order of training days during discrimination training often had more than two safe days between each danger day. The extra safe context exposure with the saccharin solution allowed the rats to learn the safe versus danger context discrimination without generalizing the avoidance of consumption to the safe context. The rats were videotaped during the last cycle (cycle 10). The videotapes were scored to determine the frequency with which the rats exhibited specific aversive behaviors (see below) during each 1-min interval of the 20 min trials.

Test cycles. Following acquisition, all 16 rats were given two 3-day test cycles (S-D-S) to determine the effect of the presence of saccharin solution on aversive responding. All rats were video recorded during the test cycles and no injections were given. For one of the test cycles, the rats were placed in the box, one at a time, for 20 min with the saccharin solution available for the entire trial. For the other test cycle, the rats were placed in the box, one at a time, and remained there for 20 min without the presentation of the saccharin solution. These test cycles evaluated the influence of the saccharin solution presentation on the distribution of gaping and chin rubbing over the course of the 20 min trials. The order of the two tests was counterbalanced and a single retraining cycle, identical to acquisition training, was administered between the two tests to prevent possible extinction.

Vinegar transfer test. After all rats had received both test cycles and two cycles of discrimination retraining, a two-day transfer test was conducted where the rats were given a 2% white vinegar solution (5% v/v acetic acid, Sunfresh Ltd., Toronto, ON)

rather than the training saccharin solution for the second 10 min of each trial. The transfer test evaluated the focus of the aversive and avoidance responding learned in the discrimination training; low consumption or increased aversive responding during the danger trial of the transfer test would suggest that responding is to the danger context, rather than to the saccharin solution presented in the danger context. Half of the rats received the danger condition first, while the other half received the safe condition first. The amount of vinegar solution consumed (in g) was recorded, and all transfer test trials were video recorded. No injections were given to the rats upon removal from the training boxes. A three-day retraining cycle identical to acquisition training was given to all rats after the transfer test, but these trials were not video recorded.

Extinction. After the transfer test and one cycle of discrimination retraining, the rats were given one of two extinction procedures. During the 10 extinction trials all rats were placed in the danger context for 20 min. Half of the rats (Group Box-Water) had tap water available for consumption through the danger hole for the second 10 min of the trials. The other half of the rats (Group Box) had no fluid available during extinction trials. These different extinction procedures were used to assess possible differences in associative strengths of stimuli that might not be obvious during discrimination training. The amount of tap water consumed (in g) was recorded and no injections were given. Rats were video recorded on Days 1 and 10 of extinction. Upon completion of the 10 extinction trials, the rats were given a two-day retention cycle in which all rats received saccharin solution during the second 10 min of the trial (with the order of safe and danger

days counterbalanced). The amount of saccharin solution consumed (in g) was recorded and no injections were given. The rats were video recorded during the retention cycle.

Scoring of videotaped behavior. All video recordings of trials were reviewed individually and scored using the Logger (Memorial University of Newfoundland) application. A second researcher reviewed random trial recordings and confirmed the occurrence of each behavior recorded by the main video scorer. The choice of behaviors to be examined and the definitions used were derived from the results of Brown et al. (2011), and included gaping and chin rubbing. A single gape was defined as "a large opening (and partial closing), of the rat mandible, with the corners of the mouth furled back that occur[s] in a rhythmic fashion in quick succession" (Brown et al., 2011, p. 97). A chin rub consisted of "the rat rubbing its chin either across the glass floor or the sides of the test box" (Brown et al., 2011, p. 97) starting with the rat's chin touching the surface, moving, and ending when it was lifted off.

Results

Discrimination training. All rats acquired the context discrimination, drinking less saccharin solution on the danger days than on the safe days as training progressed (Figure 1). A 10 x 3 (Cycle [1-10] x Day [S-D-S]) repeated measures analysis of variance (ANOVA) on saccharin consumption during discrimination training revealed significant main effects of Cycle, $F(9, 135) = 8.29$, $p < .05$, and Day, $F(2, 30) = 57.02$, $p < .05$, and a significant Cycle x Day interaction, $F(18, 270) = 24.68$, $p < .05$, reflecting acquisition of the discrimination. Tukey's multiple comparisons test revealed that for the first five cycles saccharin solution consumption was significantly higher on the danger day than on

the following safe day, *p*s < .05. From cycle 6 onward, consumption on the danger day was significantly lower than on each safe day, *p*s < .05.

During the last cycle of acquisition, rats gaped significantly more on the danger day than on the preceding or subsequent safe day (Figure 2A). A one-way ANOVA revealed a significant effect of Day, $F(2, 30) = 56.38$, $p < .05$, and follow-up Tukey's tests confirmed that rats gaped more on the danger day than on the two safe days, *p*s < .05), which did not differ, $p > .05$. As previously reported by Brown et al. (2011), rats gaped more during the first 10 min of the danger trial (before the saccharin solution was available) than during the second 10 min of the trial (when the saccharin solution was available) (Figure 2B). A 2 x 3 (Phase [First 10 min, Second 10 min] x Day [S-D-S]) repeated measures ANOVA revealed a significant main effect of Phase, $F(1, 15) = 10.28$, $p < .05$, and Day, $F(2, 30) = 56.38$, $p < .05$, as well as a significant interaction, $F(2, 30) = 17.38$, $p < .05$. Bonferroni's multiple comparisons test confirmed that rats gaped significantly more in the first 10 min than in the second 10 min of a danger trial, $p < .05$, but there were no differences in gaping during the first and second 10 min of the safe trials, *p*s > .05. An examination of the distribution of gaping at one-minute intervals during the last danger day revealed that gaping was initially high and then tapered off with a small increase in responding when the saccharin solution was first presented (at minute 10, Figure 2C). Due to the low number of gapes during safe trials, those data were not analyzed at one-minute intervals. A one-way ANOVA on gaping scores during the danger trial revealed a main effect of Minute, $F(19, 285) = 4.08$, $p < .05$, and a trend analysis confirmed a significant linear trend, $F(1, 15) = 28.64$, $p < .05$. This suggests that

gaping was initially high in response to the danger context and then decreased over the duration of the trial.

Chin rubbing was also more frequent during the danger trial than during the safe trials of the last cycle of acquisition (Figure 3A). A one-way ANOVA revealed a significant effect of Day, $F(2, 30) = 16.72$, $p < .05$, and follow-up Tukey's tests confirmed that chin rubbing occurred more often on the danger trial than on either of the safe trials, ps $< .05$, which did not differ, $p > .05$. In contrast to the pattern observed with gaping, the frequency of chin rubbing did not change systematically across the 20-min trial (Figure 3B, C). A 2 x 3 (Phase [First 10 min, Second 10 min] x Day [S-D-S]) ANOVA revealed a main effect of Day, $F(2, 30) = 16.22$, $p < .05$, confirming that rats exhibited more chin rubbing on the danger trial than on safe trials, but there was no significant effect of Phase. Similarly, a one-way ANOVA examining chin rub responses in one-minute intervals on the last danger day of acquisition training revealed no effect of minute, $F(19, 285) = 1.21$, $p > .05$. Again, due to the small number of chin rubs on safe days these data were not analyzed.

Test cycles. The data of interest for the test cycles are the distribution of aversive responses over the entire duration of the 20 min danger trial (Figure 4). Because aversive responding was very low on safe days, these data were not analyzed. A 2 x 20 (Test [Saccharin, No Saccharin] x Minute [1, 2, 3…20] repeated measures ANOVA of gaping responses revealed only a significant main effect of Minute, $F(19, 285) = 18.22$, $p < .05$. Trend analyses on each test cycle revealed significant linear trends for gaping responses both with, $F(1, 15) = 46.40$, $p < .05$, and without, $F(1, 15) = 47.77$, $p < .05$, saccharin

availability (Figure 4A). The distribution of chin rub responses on the danger day of the two tests was analyzed in the same way. The ANOVA revealed significant main effects of Test, $F(1, 15) = 8.31$, $p < .05$, and Minute, $F(19, 285) = 2.52$, $p < .05$. As can be seen in Figure 4B, chin rubs were more frequent during the test without the saccharin solution than during the test with saccharin solution present. However, despite a significant effect of minute, no significant linear trend was revealed for chin rubbing during either test cycle.

Vinegar transfer test. When vinegar was substituted for the trained flavor, the rats consumed more vinegar on the safe trial than on the danger trial, $t(15) = 2.75$, $p < .05$ (Figure 5A). The rats exhibited more gaping, $t(15) = 6.18$, $p < .05$ (Figure 5B) and chin rubbing, $t(15) = 6.77$, $p < .05$ (Figure 5C) in the danger context relative to the safe context on the vinegar transfer test.

As in the earlier tests, the distribution of gaping over one-minute intervals on the danger day was analyzed. A one-way ANOVA revealed a significant effect of Minute, $F(19, 285) = 3.49$, $p < .05$. Trend analysis revealed a significant linear trend, $F(1, 15) = 13.12$, $p < .05$, confirming gaping was high initially but significantly decreased during the 20 min trial (data not shown). The pattern of chin rubbing during the danger trial of the transfer test was also analyzed and the one-way ANOVA revealed a significant effect of Minute, $F(19, 285) = 2.72$, $p < .05$. However, trend analyses did not reveal any meaningful trend, suggesting that there was no consistent pattern to the distribution of chin rub responses (data not shown).

Extinction. The rats were divided into two extinction conditions: one with tap water and one without fluid. Extinction consisted of 10 exposures to the danger context only. A one way ANOVA on water consumption over the 10 extinction trials revealed a significant effect of Trial, $F(9, 63) = 5.84$, $p < .05$, and a linear trend analysis confirmed that water consumption increased over the 10 extinction trials, $F(1,7) = 7.199$, $p < .05$ (Figure 6A).

A 2 x 2 (Extinction Condition [Box, Box-Water] x Trial [1, 10]) ANOVA on gaping during the first and last extinction trial revealed only a main effect of Trial, $F(1, 14) = 35.52$, $p < .05$, confirming that rats gaped less on the tenth day of extinction than on the first day of extinction, regardless of how extinction was conducted (Figure 6B). The same two-way ANOVA on chin rubbing again revealed only a main effect of Trial, $F(1, 14) = 18.28$, $p < .05$, demonstrating that rats chin rubbed less on the tenth day of extinction than on the first day of extinction, regardless of how extinction was conducted (Figure 6C).

Retention test. Following the 10 days of extinction, all rats were given a two-day retention test with saccharin solution to drink. The extinction condition (exposure to danger context alone versus exposure to danger context plus fluid) did not influence any of the measures on the retention test (consumption, gaping, or chin rubs). A 2 x 2 (Extinction Condition [Box, Box-Water] x Context [S, D]) ANOVA analyzing saccharin solution consumption during the retention test revealed only a main effect of Context, $F(1, 14) = 12.97$, $p < .05$. Regardless of how extinction occurred, after 10 days of extinction, the rats continued to drink more saccharin solution in the safe context than in

the danger context (Figure 7A). A similar two-way ANOVA on gaping during the retention test revealed only a main effect of Context, $F(1, 14) = 7.63$, $p < .05$. More gaping occurred in the danger context than in the safe context following 10 days of extinction, regardless of extinction condition (Figure 7B). A similar two-way ANOVA on chin rubbing during the retention test revealed an effect of Context that approached significance, $F(1, 14) = 4.49$, $p = .053$ (Figure 7C).

To further assess whether the extinction procedures were effective, we compared performance on the retention test to performance on the first safe trial and danger trial of the last cycle of discrimination training (see Figure 8). A 2 x 2 x 2 (Extinction Condition [Box, Box-Water] x Cycle [Last Cycle, Retention Test] x Context [S, D]) ANOVA comparing saccharin consumption revealed main effects of Cycle, $F(1, 14) = 63.08$, $p < .05$, and Context, $F(1,14) = 50.40$, $p < .05$ (Figure 8A). While consumption increased from the last cycle to the retention test, differential consumption in the two training contexts was still evident after extinction.

A similar three-way ANOVA on gaping revealed significant main effects of Cycle, $F(1, 14) = 54.23$, $p < .05$, and Context, $F(1, 14) = 63.88$, $p < .05$, and a significant Cycle x Context interaction, $F(1, 14) = 58.07$, $p < .05$ (Figure 8B). Holm-Bonferroni follow-up tests revealed that gaping on the two safe days was similar, $p > .05$, but that gaping was lower on the danger day of the retention test than on the danger day of the last cycle, $p < .05$. Gaping was significantly higher on the danger day than on the safe day on both the last cycle and the retention test, ps $< .05$.

A similar pattern was observed when chin rubs were examined. A 2 x 2 x 2 (Extinction Condition [Box, Box-Water] x Cycle [Last Cycle, Retention Test] x Context [S, D]) ANOVA revealed significant main effects of Cycle, $F(1,14) = 14.27$, $p < .05$, and Context, $F(1, 14) = 15.66$, $p < .05$, and a significant Cycle x Context interaction, $F(1,14) = 14.27$, $p < .05$ (Figure 8C). While there was no difference between chin rubbing on the two safe days (mean = 0 in both cases), Holm-Bonferroni follow-up tests revealed that chin rubbing was lower on the danger day of the retention test than on the danger day of the last cycle, $p < .05$. Although chin rubbing was significantly higher on the danger day than on the safe day of the last cycle, $p < .05$, this difference was no longer significant on the retention test, $p > .05$.

Discussion

All rats learned the context discrimination task, consuming less saccharin solution on danger days than on safe days, with this differential consumption first appearing during Cycle 6. This timeline is comparable to other studies employing a similar discrimination paradigm (Brown et al., 2011; Murphy & Skinner, 2005; Peunte et al., 1988; Skinner et al., 1994). A suppression of saccharin solution consumption in the danger context and a lack of any aversive responding in the safe context suggests conditional control over both aversive and avoidance responses. As in other studies (i.e., Brown et al., 2011; Limebeer et al., 2008; Parker, 2003) gaping and chin rubbing were used as measures of aversion to both contextual and flavor cues.

The frequency and pattern of both gaping and chin rubbing behaviors during the last cycle of acquisition were comparable to the findings reported by Brown et al. (2011).

While gaping and chin rubbing were nearly completely absent during both safe days of the last cycle, animals exhibited a large number of both behaviors during the danger trial. However, the pattern of gaping and chin rubbing was different during the danger trial. As in the results of Brown et al., gaping was most frequent in the first 10 min of the trial and decreased noticeably in the second 10 min, while chin rubbing was more evenly distributed throughout the entire 20 min. To further examine the within-trial decrease in gaping and the difference between the patterns of gaping and chin rubbing, we assessed the incidence of the two behaviors in 1-min intervals for the last cycle and two post-training tests: one with a full 20 min of saccharin solution availability and a second 20 min test with no saccharin solution. While neither modification affected the pattern of either gaping or chin rubbing, there was an interesting difference in responding when the two tests were compared. While gaping did not differ regardless of saccharin solution availability, rats chin rubbed more in the danger context when the saccharin solution was never presented for consumption.

There are several possibilities as to why gaping decreased as the trial progressed but chin rubbing did not. The presence of gaping during the first 10 min period, prior to the introduction of the saccharin solution, suggests gaping may be a measure of anticipatory nausea that decreases when the cause of the nausea (the flavor) is introduced. Alternatively, gaping might be a measure of an aversion to the danger context that is susceptible to within-trial extinction; as exposure to the context increases (over the 20 min), aversion to the context as measured by gaping decreases. Comparatively, an even distribution of chin rubbing throughout the 20 min trial suggests that chin rubbing may be

measuring only the context aversion or the fear of the context independently of the presence of the aversive flavor. This context aversion might be bigger than the flavor aversion because the rats cannot escape from the context but they can escape from the saccharin solution simply by avoiding consumption, leading to diminished gaping but not chin rubbing in the second 10 min of danger trials.

A simpler explanation for the different patterns in gaping and chin rubbing during danger trials is response fatigue. It is possible that rats make fewer gaping responses over a 20 min trial because gaping is a fatiguing motion to make, while chin rubbing is not as fatiguing. This explanation also accounts for the difference in chin rubbing, but not gaping, seen during the test cycles with and without the saccharin solution available for consumption. If gaping becomes tiring after 10 min, the timing of flavor presentation is irrelevant to the pattern of gaping over a 20 min trial; it will always decrease.

A second interesting result observed during the test cycles was that chin rubbing was more frequent when the saccharin solution was absent for a full 20 min trial than when it was available for 20 min. There are a number of possible explanations for this difference. First, it may be that rats chin rubbed less when there was saccharin solution available for 20 min because they spent some time drinking or sniffing the drinking tube, while they had more time to chin rub when these stimuli were not present. Even though rats did not drink much in the danger context, they did drink a small amount, and spent (unmeasured) time sniffing the tube without drinking. Simple opportunity to respond may explain the difference between chin rubbing in the test cycle conditions. This explanation seems unlikely as there was no change in chin rubbing when the flavor was added at 10

min during acquisition training. A second possible explanation for increased chin rubbing when the trained flavor was not presented is that chin rubbing is also a fear response, and that rats were more afraid when they were still expecting a flavor that had frequently caused illness in the danger context than when the flavor had been present the entire time. While there is no current literature suggesting that chin rubbing occurs in response to fear, there is research that links paw treading to both fear responding (Reynolds & Berridge, 2001) and taste aversion learning (Berridge, 2000; Grill & Norgren, 1978). Fear in anticipation of a cause of illness might cause higher responding than fear in the presence of a cause of illness, similar to Fanselow and Lester's (1988) theory of predatory imminence, leading to more chin rubbing during the test cycle without saccharin solution than the test cycle with 20 min of saccharin solution. It is also possible that in the sequence of behaviors rats exhibit in response to a possibility of illness, chin rubbing is an appetitive, rather than consummatory, behavior, and would therefore occur more frequently before the cause of the illness (the saccharin solution) is presented (Domjan, 2010).

Following acquisition training rats were subjected to a transfer test in which they were exposed to a novel flavor in the safe and danger contexts. As in previous research, the context discrimination learned in acquisition was maintained with rats consuming more of the novel flavor in the safe context (Murphy & Skinner, 2005; Skinner et al., 1994). In addition, we also showed that rats continued to gape and chin rub more in the danger context than the safe context on the transfer test. This finding is not surprising given the high frequency of anticipatory gaping and chin rubbing in the danger context

before the saccharin solution presentation during acquisition. The transfer of both flavor avoidance and aversive behaviors may be indicative of a strong context aversion. Whether modulatory control over consumption would be maintained in the absence of this context aversion was assessed using extinction procedures.

Following 10 days of extinction trials, rats showed increased saccharin solution consumption, and decreased gaping and chin rubbing, in the danger context relative to the end of acquisition. Performance on the retention test (i.e., flavor avoidance and aversive measures) did not differ based on the presence/absence of water during extinction trials. In previous research, rats that were allowed to drink water during extinction no longer showed differential consumption of saccharin solution on a retention test but continued to avoid the danger context during a place choice test. In contrast, rats that underwent extinction without water (exposure to danger context alone) no longer avoided the danger context on a place choice test but continued to show differential consumption of the saccharin solution in the safe and danger contexts (Murphy & Skinner, 2005; Skinner et al., 1994). However, in these earlier studies the contexts contained many more cues (such as odor, texture, and colour) than in the current study and the boxes were larger with more room for the rats to move around and escape from an aversive stimulus. It is possible that these extra cues created a richer context representation than the single-cue context used here, and that this may have affected the pattern of results during extinction and retention. It is also possible that potential differences between the two conditions in the rate of extinction were missed because gaping and chin rubbing were only assessed at the end of extinction rather than throughout the extinction phase.

While the extinction procedures were effective, a difference between consumption and aversive responses was still evident at the end of extinction and during the retention test. While chin rubbing in the danger context had been eliminated (and gaping was significantly reduced), consumption of the trained saccharin flavor was still lower in the danger context compared to the safe context on the retention test; in other words, 10 days of extinction training reduced differential aversive responding more than differential flavor avoidance. This is in line with previous research showing that aversive responding (Cantora et al., 2006) and appetitive responding (Dwyer et al., 2009), as measured by orofacial indicators, extinguish faster than avoidance or preference responding, as measured by consumption. The extinction of differential aversive responding combined with the lingering differential fluid consumption observed after 10 extinction trials suggests that the context might be playing a modulatory role. Since the aversion to the context was significantly reduced following extinction training, but the rats still showed differential fluid consumption the context may modulate the association between the flavor and the LiCl, as suggested by Skinner et al. (1994). This modulatory role of the context may extinguish more slowly than the context aversion.

Experiment 2

Experiment 1 revealed that rats acquired a context aversion during the context discrimination training. This context aversion was evident in the anticipatory gaping and chin rubbing in the danger context relative to the safe context, both during training and in the test without a saccharin solution present. Some have argued that differential fluid consumption, especially when the discrimination transfers to a non-trained flavor as we

observed in Experiment 1, may be indicative of a context aversion (Iguchi et al., 2014; Loy et al., 1993). Whether this aversion was due to a context-LiCl association that was potentiated by the presence of saccharin during training remains to be determined. In contrast to Experiment 1, where only one training condition was used, in the present experiment we included different training conditions in an attempt to manipulate the magnitude of the context aversion. In Experiment 2, three groups of rats were trained on the context discrimination task where the danger context with paired LiCl and the safe context was paired with saline. Rats in one group were exposed to a saccharin solution in the two training contexts, as in Experiment 1. Rats in a second group were allowed access to plain water in the training contexts, while rats in the third condition did not have access to fluid in the training contexts. Skinner et al. (1994) showed that a group trained on the context discrimination task with saccharin solution showed more flavor avoidance on a transfer test than a group trained without fluid during training. The group trained with context cues and no fluid showed a bigger avoidance of the danger context on a place choice test. These findings indicate that the presence of a flavor does not potentiate the formation of a context aversions and in fact the flavor may actually overshadow the formation of the context aversion. The addition of a group trained with water also allowed us to control for the influence of the drinking behavior.

Experiment 1 also revealed that the context aversion (as measured by gaping and chin rubbing) seemed to extinguish more rapidly than the differential fluid consumption, when all three measures were assessed during the retention test at the end of extinction. This pattern was evident regardless of whether a drinking response was present on

extinction trials. Previous work has shown that flavor avoidance extinguished more rapidly if rats were allowed access to fluid during extinction than if they were given exposure to the danger context alone (Murphy & Skinner, 2005). In addition, exposure to the danger context alone produced better extinction of the context aversion, which in that study was assessed by context avoidance on a place choice test. As in Experiment 1, in the present experiment we examined extinction using two conditions: one that involved exposure to the danger context with fluid and one that involved exposure to the danger context alone. The extinction phase lasted longer in Experiment 2 and the effects of extinction on consumption of the training solution and context aversion were assessed at multiple times rather than simply at the end of extinction. In addition, place choice tests were also included as a measure of context aversion/avoidance, as in the earlier work by Murphy and Skinner (2005).

Method

Subjects. Thirty-six male Long-Evans rats, obtained from the Charles River Company (St. Constant, Quebec, Canada) and weighing between 136g and 161g at the start of training, were used. The rats were maintained as in Experiment 1. Animal care and all procedures used in the experiment were approved by Memorial University‘s Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Apparatus. Six wooden rectangular boxes identical to those in Experiment 1 were used. The table and video camera setup were also identical with the exception of the use of six 60 W lights placed under the table.

For the place choice test, a rectangular wooden box with no bottom (inner dimensions 85 x 25.5 x 38 cm) painted black and white on opposite sides (inner dimensions 25.5 x 35.75 cm) and separated by a neutral grey area (25.5 x 12.75 cm), was used. The place choice box was placed on the same glass table described above with similar lighting conditions to allow for video recording from below. In contrast to the training boxes, this box did not have a top so ceiling lights were turned off during the place choice tests.

During training, rats in groups receiving fluid were given access to either tap water or a 0.1% saccharin solution. Upon completion of training trials, all rats were given an intra-peritoneal (ip) injection of either saline (0.9% NaCl; 3.0 ml/kg) or lithium chloride (LiCl) (0.47 M; 3.0 ml/kg).

Discrimination training. Rats were trained on a context discrimination task similar to that described in Experiment 1. On safe days, the rats were placed in either a black or white box for a 10 min period during which no fluid (Group Box, n = 12), tap water (Group Box-Water, n = 12), or a saccharin solution (Group Box-Sacc, n = 12) was available for consumption through a metal drinking tube in the hole on the short wall of the training box. A 10 min trial duration was used rather than the 20 min trial duration used in Experiment 1 following the observation that the majority of the behavioral responding took place during the first 10 min of those trials (see Figure 2). Since gaping was highest in the first 10 min and there was little difference between chin rubbing in the first 10 min and the second 10 min of Experiment 1, the trials lasted for 10 min rather than 20 min with complete overlap of the context and the fluid. Upon removal from the

box and fluid (if available) the rats were given an i.p. injection of saline and returned to their home cages. On danger days, the rats were placed in the alternate context for 10 min. Removal from the box and the fluid (if available) was followed by an ip injection of LiCl and the rats were returned to their home cages. Half of the rats were placed in the white box on safe days and the black box on danger days, and the other half received the opposite contingency. The amount of water or saccharin solution consumed (in g) each day was recorded. The full sequence of training and tests administered can be seen in Table 1.

The acquisition phase was divided into nine 3-day cycles (S-D-S) and the rats were videotaped during cycles 3, 6 and 9. The videotapes were scored to determine the frequency with which the rats exhibited specific aversive behaviors (as in Experiment 1) during each 1 min interval of the 10 min trials.

Place choice test. Immediately following cycle 9, and again following the 10th and 19th days of extinction, the rats were given a place choice test (PCT) in which they were placed in the neutral grey area of the place choice box and allowed to move freely around the box for 10 min. The time (in s) spent in the safe and danger contexts was recorded for each rat in real time from a television (42” LCD, LG) displaying the video feed from the camera under the box. Sessions were also video recorded for confirmation of results. A 3-day retraining cycle identical to acquisition followed the PCT (see Table 1 for a description of the order in which these tests were administered).

Vinegar transfer test. After the PCT and one cycle of retraining, all rats were given a 2-day transfer test in which a 2% white vinegar solution (5% v/v acetic acid,

Sunfresh Ltd., Toronto, ON) was available for consumption for 10 min regardless of training condition. Half of the rats in each group received the safe condition first, and half received the danger condition first. No injections were given to the rats following removal from the boxes. The amount of vinegar solution consumed (in g) was recorded, and all trials were video recorded.

Retention. Following the transfer test, all rats were given a 2-day retention cycle in which fluid was presented for consumption according to their training condition. Half of the rats in each group received the safe condition first, and half received the danger condition first. No injections were given to the rats following removal from the boxes. The amount of water or saccharin solution consumed (in g) was recorded and all trials were video recorded. Upon completion of the retention test the rats were given one cycle of retraining identical to initial acquisition, and this 3-day cycle (S-D-S) was video recorded.

Extinction. After one cycle of discrimination retraining, the rats were given one of two extinction procedures. During extinction trials, rats were placed in the danger context for 10 min either in the presence or absence of fluid (unsweetened cherry Kool-Aid). This resulted in six extinction groups (n = 6; where FE indicates that there was fluid present during extinction trials and NFE indicates that there was no fluid present during extinction trials): Group Box-FE; Group Box-NFE; Group Box-Water-FE; Group Box-Water-NFE; Group Box-Sacc-FE; and Group Box-Sacc-NFE. As in Experiment 1, these different extinction procedures were used to assess possible differences in associative strengths of stimuli that might not be obvious during discrimination training. Rats were

matched for fluid consumption during discrimination training, transfer, and retention tests as well as place test performance prior to the onset of extinction. Extinction continued for 19 trials, with video recording taking place on days 1, 4, 7, 10, 13, 16, and 19. Kool-Aid consumption (in g) was recorded each day and no injections were given following removal from the boxes.

Post extinction tests. Place choice tests and retention cycles (described above) were given following trials 10 and 19 of extinction. Rats in Group Box that did not receive fluid during acquisition were given a 2% vinegar solution identical to that used in the transfer test during the second and third retention tests to allow for a comparison of consumption across extinction groups. All tests were video recorded.

Scoring of videotaped behavior. Video recordings were reviewed as in Experiment 1.

Results

Discrimination training. All rats acquired the discrimination between the safe and danger contexts, with rats in Groups Box-Water and Box-Sacc consuming less fluid in the danger context than in the safe context as training progressed (Figure 9). A 2 x 9 x 3 (Group [Box-Water, Box-Sacc] x Cycle [1, 2, 3, … 9] x Context [S-D-S]) ANOVA revealed main effects of Cycle, $F(8,176) = 2.871$, $p < .05$, and Context, $F(2, 44) = 212.323$, $p < .05$, as well a significant Group x Context interaction, $F(2, 44) = 6.543$, $p < .05$, and a significant Cycle x Context interaction, $F(16, 352) = 31.945$, $p < .05$, reflecting the acquisition of the discrimination. Rats in Group Box-Water began consuming less on the danger day than on the preceding safe day in Cycle 3, $t(11) = 3.199$, adjusted $p < .05$,

while increased consumption on the second safe day of the cycle (following a danger trial) began in Cycle 4, $t(11) = -5.922$, adjusted $p < .05$. Rats in Group Box-Sacc began consuming less on the danger day than on the preceding safe day in Cycle 4, $t(11) = 3.154$, adjusted $p < .05$, while increased consumption on the second safe day (following a danger trial) began in Cycle 5, $t(11) = -4.834$, adjusted $p < .05$. Analysis of consumption data during acquisition was not possible for Group Box.

During the initial discrimination training, rats in all groups showed a similar pattern of gaping and chin rubbing responses. As seen in Figure 10, responding for each behavior was very low on all days during cycle 3 (panels A and B), and high on danger days and low on safe days by cycle 9 (panels E and F). A 3 x 3 x 3 (Group [Box, Box-Water, Box-Sacc] x Cycle [3, 6, 9] x Context [S-D-S]) ANOVA of gaping during discrimination training revealed significant main effects of Cycle, $F(2, 66) = 63.156$, $p < .05$, and Context, $F(2, 66) = 55.029$, $p < .05$, as well as a significant Cycle x Context interaction, $F(4, 132) = 32.101$, $p < .05$. Follow-up Tukey's tests on the significant interaction showed that gaping on the danger day was higher than on each of the safe days on cycles 6 (Figure 10C) and 9 (Figure 10E), while gaping on the safe days did not differ, ps < .05. Similarly, a 3 x 3 x 3 (Group [Box, Box-Water, Box-Sacc] x Cycle (3, 6, 9] x Context [S-D-S]) ANOVA of chin rubbing during discrimination training revealed significant main effects of Cycle, $F(2, 66) = 12.547$, $p < .05$, and Context, $F(2, 66) = 15.835$, $p < .05$, as well as a significant Cycle x Context interaction, $F(4, 132) = 8.156$, $p < .05$. Post-hoc Tukey's tests showed that chin rubbing was significantly higher on the danger day compared to the safe days during cycle 9 (Figure 10F), but chin rubbing on

safe days did not differ, *p*s < .05. Thus, differential chin rubbing in the two contexts did not emerge until later in training, after cycle 6. While it is hard to discern when differential gaping in the two contexts began to emerge, it appears that differential responding to the safe and danger contexts began at approximately the same time for consumption (around Cycle 4) and gaping (prior to Cycle 6), but that differential chin rubbing emerged in the latter part of training.

Place choice test. Following discrimination training, a PCT was administered as a measure of context avoidance in each group. The groups displayed an equal avoidance of the danger context, spending more time in the safe than danger side of the choice box (Figure 11). A 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Context [S, D]) ANOVA revealed only a main effect of Context, $F(1, 33) = 24.51$, $p < .05$, suggesting that training condition did not affect context avoidance unlike in the Murphy and Skinner (2005) study.

Vinegar transfer test. Training condition also did not affect responding to a novel flavor on the transfer test given immediately after the PCT (see Figure 12, left column). Analyses via 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Context [S, D]) ANOVAs showed that differential responding to the safe and danger contexts was maintained when measuring consumption, $F(1,33) = 88.65$, $p < .05$, (Figure 12A), gaping, $F(1,33) = 95.15$, $p < .05$, (Figure 12C), and chin rubbing, $F(1, 33) = 51.15$, $p < .05$, (Figure 12E). As with initial acquisition, no group differences were found in any of the measures, in contrast to the Murphy and Skinner (2005) study.

Retention test. A two-day retention test using the original training fluid for each group was conducted following the transfer test (Figure 12, right column). The discrimination was retained as assessed by the consumption, gaping, and chin rubbing measures. A 2 x 2 (Group [Box-Water, Box-Sacc] x Context (S, D) ANOVA assessing consumption revealed only a significant main effect of Context, $F(1, 22) = 338.1$, $p < .05$ (Figure 12B). A 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Context (S, D) ANOVA of gaping showed only a main effect of Context, $F(1, 33) = 88.631$, $p < .05$, (Figure 12D).

When chin rubbing during the retention test was analysed (Figure 12F), a 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Context [S, D]) ANOVA revealed main effects of Group, $F(2, 33) = 3.82$, $p < .05$, and Context, $F(1, 33) = 43.60$, $p < .05$, as well as a significant interaction, $F(2, 33) = 3.54$, $p < .05$. Follow-up independent samples t-tests on chin rubbing in the danger context during the retention test showed that rats in Group Box chin rubbed more than rats in Group Box-Water, $t(22) = 2.541$, $p < .05$, while the difference in chin rubbing by rats in Group Box and those in Group Box-Saccharin approached significance, $t(22) = 2.051$, $p = .052$. This contrasts with acquisition training, where no group differences were found.

A comparison of the data from Cycle 9 and the first Retention test suggests that there might have been some extinction of the chin rubbing response, particularly in the groups that had access to fluids during training (Groups Box-Water and Box-Sacc). A 3 x 2 x 2 (Group [Box, Box-Water, Box-Sacc] x Test [Cycle 9, Retention 1] x Context [S, D]) ANOVA was conducted to test if there was any extinction of chin rubbing following the transfer test. The ANOVA revealed main effects of Test, $F(1, 33) = 6.597$, $p < .05$,

and Context, $F(1, 33) = 59.289$, $p < .05$, and an effect of Group that approached significance, $F(2, 33) = 3.192$, $p = .054$. There was also a significant Test x Context interaction, $F(1, 33) = 5.231$, $p < .05$. Holm-Bonferroni post-hoc tests showed that while rats still chin rubbed more in the danger context than the safe context on the retention test, they chin rubbed less on the danger day of the retention test than on the danger day of the last cycle, adjusted ps < .05. In particular, rats in Group Box-Water chin rubbed less during the danger trial of the retention test than they did during the danger trial of Cycle 9 of acquisition training, $t(11) = 4.335$, $p < .05$.

Extinction. Following a three-day retraining cycle, all rats were given extinction trials that included exposure to only the danger context. Half the rats were extinguished in the presence of fluid (unsweetened cherry Kool-Aid) and half were extinguished without fluid. For those rats extinguished in the presence of fluid, the first day of extinction was the equivalent of a transfer test to a novel fluid. While the first transfer test revealed no group differences, an examination of Figure 13 suggests that there were group differences on the first day of extinction. A 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Test [Transfer Danger, Extinction Day 1]) ANOVA comparing consumption on the danger day of the transfer test and the first day of extinction revealed a significant main effect of Test, $F(1, 15) = 15.629$, $p < .05$, and a significant Group x Test interaction, $F(2, 15) = 4.38$, $p < .05$. Paired-samples t-tests showed that rats in Group Box consumed more on the first extinction trial than on the danger trial of the transfer test, $t(5) = -3.737$, $p < .05$, while Groups Box-Water and Box-Sacc consumed equal amounts on both tests. Thus, rats in Group Box showed less transfer of flavor avoidance on the first day of

extinction compared to the transfer test, while rats in Groups Box-Water and Box-Sacc showed a similarly strong transfer of flavor avoidance on both trials, a finding that is in line with previous research (Murphy & Skinner, 2005). It is possible that there was a floor effect present during the transfer test, preventing group differences from appearing due to the strength of the context-LiCl associations present in all groups.

Throughout the 19 days of extinction, rats in the three subgroups given fluid gradually increased the amount of Kool-Aid consumed (Figure 13). A 3 x 19 (Group [Box, Box-Water, Box-Sacc] x Day [1, 2, 3, … 19]) ANOVA revealed only a main effect of Day, $F(18, 270) = 16.83$, $p <. 05$. However, examination of Figure 13 suggested that the differences in consumption noted on day 1 (Group Box consumed more than Groups Box-Water and Box-Sacc) were maintained for the first 10 days of extinction. A 2 x 10 (Group [Box, Box-Water and Box-Sacc combined] x Day [1, 2, 3, … 10]) ANOVA comparing consumption in Group Box to consumption in the two groups that had received fluid during training revealed significant main effects of both Group, $F(1, 16) = 7.550$, $p < .05$, and Day, $F(1, 16) = 35.607$, $p < .05$. Until day 10 of extinction, rats that received water or saccharin solution during discrimination training drank less than rats that did not receive fluid in their original training.

Aversive responding was examined every third day of extinction to determine the rate of extinction of gaping and chin rubbing. To compare the effects of the two extinction procedures (access or no access to Kool-Aid), gaping throughout extinction was examined by both training group (Box, Box-Water, or Box-Sacc) and extinction condition (FE or NFE). For all training groups, gaping was high on the first day of

extinction and decreased regardless of the presence of fluid during the extinction trials (Figure 14, left column). A 3 x 2 x 8 (Group [Box, Box-Water, Box-Sacc] x Extinction Condition [NFE, FE] vs Extinction Trial [1, 4, 7, … 19]) ANOVA of gaping during extinction training revealed main effects of Group, $F(2, 30) = 3.919$, $p < .05$, and Extinction Trial, $F(6, 180) = 59.746$, $p < .05$. Follow-up Holm-Bonferonni tests showed that rats gaped more on the first extinction trial than the fourth, and more during the 10th extinction trial than the 13th, adjusted ps < .05, and Tukey's HSD confirmed that rats in Group Box (Figure 14A) gaped more than rats in Group Box-Sacc, $p < .05$ (Figure 14E). Gaping on the first day of extinction was also compared to gaping in the danger context on the transfer test since both trials can be considered transfer tests. A 3 x 2 (Group [Box, Box-Water, Box-Sacc] x Test [Transfer Danger, Extinction 1]) ANOVA revealed a significant main effect of Test, $F(1, 33) = 9.172$, $p < .05$, and a follow-up paired-samples t-test showed that gaping was more frequent during the danger trial of the Transfer test than on the first day of extinction training, $t(35) = 3.082$, $p < .05$.

Similarly, chin rubbing was initially high at the start of extinction and then decreased in frequency (Figure 14, right panel). A 3 x 2 x 8 (Group [Box, Box-Water, Box-Sacc] x Extinction Condition [NFE, FE] vs Extinction Trial [1, 4, 7, … 19]) ANOVA of chin rubbing during extinction training revealed a main effect of Trial, $F(6, 180) = 49.022$, $p < .05$, a significant Extinction Condition x Trial interaction, $F(12,180) = 3.409$, $p < .05$, and a Group x Extinction Condition x Trial interaction, $F(12, 180) = 2.078$, $p < .05$. Follow-up Holm-Bonferroni tests showed that rats chin rubbed more frequently in extinction trial 1 than in trial 4, in trial 4 than in trial 7, and in trial 7 than in

trial 10, adjusted *p*s < .05. Despite interaction effects involving training groups and extinction conditions, these comparisons were not found to be significant in post-hoc testing. However, Figure 14B suggests that rats in Group Box-NFE chin rubbed the most on the first trial of extinction. This result is similar to the high level of chin rubbing observed during the test cycle without saccharin solution in Experiment 1.

Post extinction tests.

Place choice tests. In addition to the PCT given after acquisition, rats were given retests after 10 and 19 days of extinction. Generally, it was found that rats spent less time in the danger context than the safe context after acquisition training and 10 extinction trials (PCT 1 and 2, respectively), but that effect disappeared by 19 days of extinction (PCT 3). Within Group Box (Figure 15, left panel), a 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed a main effect of Context, $F(1, 10) = 5.405$, $p < .05$, and a Test x Context interaction, $F(2, 20) = 7.660$, $p < .05$. Follow-up Holm-Bonferroni tests revealed that rats spent more time in the safe context than the danger context on the first PCT (Figure 15A), and that rats spent more time in the danger context during the third PCT (Figure 15G) than the second PCT (Figure 15D), adjusted *p*s < .05. Rats in Group Box, that were trained without access to fluid, no longer avoided the danger context relative to the safe context following 10 days of extinction (Figure 15D), and spent even more time in the danger context after 19 extinction trials (Figure 15G).

Rats in group Box-Water responded similarly (Figure 15, middle panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed a

main effect of Context, $F(1, 10) = 5.920$, $p < .05$, and a Test x Context interaction, $F(2, 20) = 4.670$, $p < .05$. Follow-up Holm-Bonferroni tests showed that rats spent more time in the safe context than the danger context on the first PCT (Figure 15B), and that rats spent more time in the danger context on the third PCT (Figure 15H) than the second PCT (Figure 15E), adjusted ps < .05. Additionally, the time spent in the safe and danger contexts of the second PCT approached a significant difference, adjusted $p = .052$, suggesting that the context avoidance might have extinguished somewhat more slowly in Group Box-Water than in Group Box.

Interestingly, when divided into sub-groups based on extinction condition, rats in Group Box-Sacc did not show any significant differences in the time spent in each context during any of the PCTs (Figure 15, right panel). There was no difference in time spent in the danger context relative to the safe context prior to extinction trials in this group (Figure 15C), and rats did not spend any more time in the danger context after 19 days of extinction (Figure 15I) than they did before extinction started. This contrasts with Groups Box and Box-Water that showed a measurable context aversion following acquisition training, which was extinguished after 10-19 extinction trials. The lack of a context avoidance by only Group Box-Sacc suggests that the flavor cue may have overshadowed the context cue, similar to the findings of Skinner et al. (1994).

Retention. After 10 and 19 days of extinction, 2-day retention tests were conducted to measure the effectiveness of the two extinction procedures. These tests were compared to the identical retention test administered after the transfer test prior to the start of extinction (see Table 1). For Group Box, a vinegar solution was used in the

retention tests and these trials were compared to the transfer test, where Group Box also received a vinegar solution. Consumption data showed that flavor avoidance was eliminated in Group Box after 10 days of extinction trials, regardless of the presence of fluid during extinction (Figure 16, left panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA showed a main effect of Test, $F(2,20) = 65.880$, $p < .05$, and a Test x Context interaction, $F(2, 20) = 9.897$, $p < .05$. Follow-up Holm-Bonferroni tests showed that rats consumed less in the danger context than in the safe context on the first retention test (Figure 16A), but that consumption was similar in both contexts during the second (Figure 16D) and third (Figure 16G) retention tests, which did not differ from each other, adjusted *p*s < .05. In Group Box, the flavor avoidance was no longer present after 10 days of extinction regardless of the presence or absence of KoolAid during the extinction trials.

Differential consumption in rats in Group Box-Water was also eliminated after 10 extinction trials (Figure 16, middle panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test, $F(2, 20) = 16.685$, $p < .05$, and Context, $F(1, 10) = 145.013$, $p < .05$. There was also a significant Extinction Condition x Test interaction, $F(2, 20) = 3.503$, $p = .05$, a significant Extinction Condition x Context interaction, $F(1, 10) = 277.28$, $p < .05$, and significant Context x Test interaction, $F(2, 20) = 12.135$, $p < .05$. Follow-up Holm-Bonferroni tests showed that rats consumed more in the safe than danger context during the first (Figure 16B) and third (Figure 16H), but not second (Figure 16E), retention tests, and that consumption was higher in the danger context during the second retention test than in the first, adjusted *p*s

< .05. Several extinction condition differences were also found with post hoc tests. First, consumption by rats in Group Box-Water-FE was higher in the danger context during the second retention test than in the first; second, during the second retention test, rats in Group Box-Water-FE consumed more in the danger context than rats in Group Box-Water-NFE, adjusted *p*s < .05. Additionally, rats in Group Box-Water-NFE consumed more in the second retention test than in the first (see Figure 16), and this comparison approached significance, adjusted $p = .056$. The return of differential consumption after 19 extinction trials appeared to be due to an increase in consumption in the safe context, rather than any decrease in consumption in the danger context. Additionally, rats that did not receive KoolAid during extinction appeared to increase their consumption in the danger context more slowly during subsequent retention tests than rats that did receive KoolAid, similar to results observed by Murphy and Skinner (2005).

Rats in Group Box-Sacc took longer to extinguish the flavor avoidance (Figure 16, right panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA of consumption data for Group Box-Sacc revealed main effects of Test, $F(2,20) = 7.807$, $p < .05$, and Context, $F(1, 10) = 64.355$, $p < .05$, as well as a Test x Context interaction, $F(2, 20) = 24.788$, $p < .05$, and a significant Extinction Condition x Test x Context interaction, $F(2, 20) = 6.343$, $p < .05$. Follow-up Holm-Bonferroni tests showed that consumption was higher in the safe context than in the danger context during the first (Figure 16C) and second (Figure 16F) retention tests, and that rats in Group Box-Sacc-NFE consumed more in the danger context of the third retention test than the second, while rats in Group Box-Sacc-FE consumed more in the danger context of the

second retention test than the first, adjusted *p*s < .05. In contrast to rats in Group Box and Group Box-Water, which did not exhibit differential consumption after 10 days of extinction training, rats in Group Box-Sacc maintained differential consumption after 10 extinction trials but not after 19 trials. Rats in Group Box-Sacc-FE displayed a different pattern of extinction of the flavour avoidance than rats in Group Box-Sacc-NFE. Rats in Group Box-Sacc-NFE did not significantly increase consumption in the danger context until some time after 10 extinction trials, while rats in Group Box-Sacc-FE showed some extinction of flavor avoidance in the danger context after 10 extinction trials, but no additional extinction following 19 trials.

Overall, gaping in all groups decreased substantially following 10 days of extinction trials and further decreased by 19 extinction trials. Within Group Box, gaping was extinguished at the second Retention test following 10 days of extinction training (Figure 17, left panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA of gaping exhibited by Group Box revealed main effects of Test, $F(2, 20) = 27.914$, $p < .05$, and Context, $F(1, 10) = 50.893$, $p < .05$, as well as a Test x Context interaction, $F(2, 20) = 25.357$, $p < .05$. Follow-up Holm-Bonferroni tests showed that gaping during the first (Figure 17A) and third (Figure 17G) retention tests was less frequent in the safe context than in the danger context, and that gaping in the danger context was less frequent during the second retention test than the first, adjusted *p*s < .05. While rats in Group Box gaped less often after 10 extinction trials, gaping did not continue to decrease with more trials regardless of extinction condition, and differential responding was still observed at the end of extinction. However, the

frequency of gaping did decrease substantially (from a mean of 12.250 (SD = 6.730) in the danger context of the first retention test to a mean of 1.333 (SD = 1.155) in the danger context of the third retention test) and significant extinction in aversive responding did occur.

Gaping by rats in Group Box-Water was still evident after 10, but not after 19, extinction trials (Figure 17, middle panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test, $F(2, 20) = 29.297$, $p < .05$, and Context, $F(1, 10) = 33.482$, $p < .05$, as well as significant Extinction Condition x Test, $F(2, 20) = 4.813$, $p < .05$, Test x Context, $F(2, 20) = 15.542$, $p < .05$, and Extinction Condition x Test x Context, $F(2, 20) = 3.838$, $p < .05$, interactions. Post hoc Holm-Bonferroni tests showed only that rats gaped less frequently during the safe trial than the danger trial of the first (Figure 17B) and second (Figure 17E) retention tests, adjusted *p*s < .05. Rats in Group Box-Water showed differential gaping after 10 extinction trials, but not after 19 extinction trials, regardless of extinction condition.

Rats in Group Box-Sacc were similar to those in Group Box-Water in that they continued to gape more in the danger than safe context after 10 extinction trials, but not after 19 trials (Figure 17, right panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test , $F(2, 20) = 12.467$, $p < .05$, and Context, $F(1, 10) = 18.962$, $p < .05$, as well as a Test x Context interaction, $F(2, 20) = 16.301$, $p < .05$. Follow-up Holm-Bonferroni tests showed that rats gaped less often in the safe context than the danger context during both the first (Figure 17C) and second (Figure 17F) retention tests, and that gaping was less frequent in the

danger context of the second retention test than the first, adjusted *p*s < .05. While rats continued to demonstrate differential responding after 10 days of extinction, gaping had dropped substantially by that point, and by 19 extinction trials there was no differential gaping observed regardless of extinction condition (Figure 17I).

Chin rubbing responding was also examined in the same way as gaping. Rats in Group Box no longer chin rubbed more in the danger context than the safe context during the second retention test, following 10 extinction trials (Figure 18, left panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test, $F(2, 20) = 24.223$, $p < .05$, and Context, $F(1, 10) = 24.516$, $p < .05$, as well as a significant Test x Context interaction, $F(2, 20) = 26.009$, $p < .05$. Follow-up Holm-Bonferroni tests showed that chin rubbing was less frequent in the safe than in the danger context during the first retention test (Figure 18A), and that chin rubbing was less frequent in the danger context during the second retention test than the first, adjusted *p*s < .05. Differential responding of chin rubbing was eliminated after 10 extinction trials regardless of whether rats received KoolAid during extinction (Figure 18D).

Chin rubbing exhibited by Group Box-Water took longer to extinguish, with rats chin rubbing more in the danger context than in the safe context on the first and second retention tests (Figure 18, middle panel). A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test, $F(2, 20) = 11.620$, $p < .05$, and Context, $F(1, 10) = 14.552$, $p < .05$, as well as a significant Test x Context interaction, $F(2, 20) = 11.403$, $p < .05$. Follow-up Holm-Bonferroni tests showed that chin rubbing was more frequent in the danger context than the safe context during the

first (Figure 18B) and second (Figure 18E) retention tests, that chin rubbing was higher in the danger context of the first retention test than the second, and that chin rubbing was higher in the danger context of the second retention test than the third (Figure 18H), adjusted *p*s < .05. Regardless of extinction condition, differential responding of chin rubbing continued after 10 days of extinction trials but was eliminated after 19 trials, and responding decreased significantly in the danger context of each retention test.

Chin rubbing in Group Box-Sacc (Figure 18, right panel) was highly variable. A 2 x 3 x 2 (Extinction Condition [NFE, FE] x Test [1, 2, 3] x Context [S, D]) ANOVA revealed main effects of Test, $F(2, 20)= 6.497$, $p < .05$, and Context, $F(1, 10) = 6.810$, $p < .05$, as well as a significant Test x Context interaction, $F(2, 20) = 6.193$, $p < .05$. However, post hoc Holm-Bonferroni tests revealed no significant differences, and this is likely due to the high variance seen in rats during the danger trial of the first retention test. It is clear, however, that differential responding of chin rubbing is eliminated in Group Box-Sacc by 10 days of extinction trials regardless of extinction condition.

Discussion

All rats acquired the context discrimination, with no group differences observed in either differential fluid consumption or differential aversive responding in the training phase. Starting at approximately Cycle 4 of acquisition, rats in Group Box-Water and Group Box-Sacc consumed less fluid in the danger context than in the safe context, slightly earlier than the onset of differential responding in Experiment 1 or in earlier similar studies (Brown et al., 2011; Murphy & Skinner, 2005). It is possible that earlier avoidance and aversive responding was observed in this experiment because of the lack

of a pre-fluid phase as has previously been employed. The 10-min trial with complete overlap of the flavor and context cues that we used may have resulted in rats learning the context discrimination earlier than in other experiments where there was some exposure to the context alone before fluid presentation (for example, Experiment 1; Brown et al., 2011).

Chin rubbing and gaping behaviors were only recorded on every third cycle during discrimination training, making it more difficult to pinpoint exactly when these behaviors began and when rats began to demonstrate differential responding. While some gaping and chin rubbing was observed in Cycle 3, higher levels of aversive responding in the danger context than in the safe context were present by Cycle 6 for gaping and Cycle 9 for chin rubbing. As with consumption results, no group differences in aversive behaviors were observed during discrimination training. The lack of group differences between training conditions may suggest that the context, rather than the opportunity to drink either water or a novel flavor, is the most important factor in the development of aversive responding. As with Experiment 1, the danger context is the best predictor of illness, while the additional water or saccharin stimuli present during the training of Group Box-Water and Group Box-Sacc are cues that are associated with illness only occasionally. Alternatively, there may be group differences during acquisition training, either in terms of the type of association or in the rate of acquisition, but the acquisition procedure was not sensitive enough to detect those differences. Comparing the groups on various measures during extinction was another way to assess possible group differences in the strength and nature of the association.

As with the results of the discrimination training, there were no group differences observed during either the Transfer Test or the first PCT, suggesting that the presence of a fluid or flavor does not potentiate the context aversion. This is contrary to the results of previous research (Skinner et al., 1994) and contrary to our expectations that rats in Group Box would exhibit the weakest transfer of fluid consumption (given that the Transfer Test was the first time this group was exposed to fluid in the training contexts) and the strongest avoidance of the danger context, while rats in Group Box-Sacc would exhibit the strongest transfer of flavor avoidance and the weakest context avoidance. However, the weakest transfer of fluid consumption might also be indicated by the fastest extinction of the flavor avoidance, as was observed in Group Box. Additionally, while there were no statistically significant differences between any of the groups during the first PCT, Group Box-Sacc never showed a preference for the safe or danger context during any PCT when this group was analysed independently. Additionally, some group differences noted during the first day of extinction training (procedurally identical to a danger trial of a transfer test) did conform to prior research findings, with Group Box showing higher consumption of Kool-Aid compared to rats in Groups Box-Water and Box-Sacc combined. The faster extinction of the flavor avoidance and increased consumption on the first extinction trial of rats in Group Box, along with the lack of danger context avoidance by rats in Group Box-Sacc, suggests that there is evidence for a weaker avoidance of the flavor by rats trained initially without fluid, and a stronger avoidance of the flavor with no avoidance of the danger context by rats trained initially with saccharin solution.

A two-day Retention test was administered following the Transfer test to see if any extinction of differential responding had occurred. During this procedure, all rats received one safe and one danger trial identical to trials during acquisition training. While rats still consumed less and showed higher aversive responding in the danger context than in the safe context during this test, rats in Group Box-Water and Group Box-Sacc consumed more on the danger trial of the Retention Test than the danger trial of Cycle 9 of acquisition training. Similarly, rats in all groups gaped and chin rubbed less during the danger trial of the Retention Test than during the danger trial of Cycle 9 of acquisition training. While this was not an expected result, decreased aversive and avoidance responding across all groups and measures suggests that some extinction likely took place during the PCT and Retention Test.

Following a retraining cycle, all rats began the extinction procedure in which half of the rats in each group were presented with a novel flavor for consumption and the other half were not presented with any fluid for consumption (resulting in six extinction groups; see Table 1). As discussed above, the first day of this procedure can be considered a second transfer test for those rats that received Kool-Aid during extinction, as a novel flavor was presented in the danger context. Consumption results from this first day of extinction training showed that rats in Group Box consumed more than rats in either Group Box-Water or Group Box-Sacc. This combined-groups difference (that is, Group Box vs Group Box-Water plus Group Box-Sacc) persisted throughout the first 10 days of extinction training, with rats in Group Box consuming more Kool-Aid than those in Groups Box-Water and Box-Sacc. While there were no group differences in

consumption by the end of extinction training, the increased consumption levels for Group Box relative to those of Group Box-Water plus Group Box Sacc during the first 10 extinction trials suggests that the availability of fluid during discrimination training does have an effect on the extinction of taste avoidance.

Rats also underwent PCTs following acquisition and during extinction training. While rats in Group Box and Group Box-Water displayed an avoidance of the danger context that was later extinguished, rats in Group Box-Sacc never had a strong avoidance of the danger context. The rats that received a flavour during acquisition did not show an avoidance of the danger context before extinction started, and did not increase the time spent in the danger context after extinction trials ended, as was observed in Group Box and Group Box-Water. This suggests that the presence of a flavour during acquisition does not potentiate a context-LiCl association (Skinner et al., 1994), but may in fact overshadow such an association.

One of the goals of this experiment was to establish how consumption of water, a novel flavor, or no fluid during discrimination training affected the rats' aversion to the context and avoidance of the fluid. These training group differences were apparent in several results. First, rats in Group Box-Sacc, where a novel flavor was presented with the safe and danger contexts, never displayed an avoidance of the danger context, and took longer for differential consumption to extinguish. A stronger flavor cue created a stronger avoidance of that flavor and a weaker avoidance of the context where illness occurred. Second, gaping was slower to extinguish in Groups Box-Water and Box-Sacc than in Group Box. While rats in Group Box did not show differential responding of

gaping following 10 days of extinction training, rats in Group Box-Water and Group Box-Sacc still gaped more in the danger context than in the safe context following the same number of extinction trials. The gaping behavior therefore seems stronger when the opportunity to consume a fluid is paired with an aversive stimulus, even if that fluid is not novel, suggesting that gaping may be more related to nausea compared to the other behaviors measured. It should be noted that there was no difference in gaping during the tests cycles of Experiment 1 where saccharin solution presentation was manipulated, as would be expected with this explanation. However, all rats in Experiment 1 were trained with saccharin solution compared to the three different training groups used in Experiment 2, which may have influenced the nature of the association between gaping, fluid consumption, and the danger context.

Another purpose of this experiment was to examine how the presence of a novel flavor during extinction affected the extinction of avoidance and aversive responding. While our findings did not match those of previous experiments using similar designs (Murphy & Skinner, 2005), we did observe some effects of extinction condition. First, the presence or absence of unsweetened KoolAid had no effect on the extinction of the rats' avoidance of the danger context as measured by the PCTs. This is an interesting contrast to the lack of any context avoidance observed in rats in Group Box-Sacc, who had access to a novel flavor during discrimination training; while pairing a novel flavor with illness during discrimination training overshadowed the association between the context and illness, a novel flavor during extinction training had no effect on the extinction of a context avoidance, probably due to the lack of an initial association between the context

and a flavor. Second, rats in both Groups Box-Water-FE and Box-Sacc-FE increased their consumption in the danger context faster than rats in Groups Box-Water-NFE and Box-Sacc-NFE when comparing consumption during the retention tests. When water or saccharin solution was available during discrimination training, avoidance of the trained fluid extinguished faster when a novel fluid was available during extinction training. Third, the analysis of gaping by rats in Group Box-Water during the retention tests showed several interactions involving extinction condition. While none of the post hoc analyses returned significant differences, it is possible that more statistical power (achieved through larger extinction group sizes) would help elucidate the extinction condition group differences detected by the ANOVA. Finally, there were no group differences found at all between rats in Group Box-FE and Group Box-NFE. This lack of effect of the availability of KoolAid during extinction on rats that were trained without a fluid, combined with the observation of several extinction condition effects in rats in the other two discrimination training groups that were trained with fluid, suggests that fluid availability during training is more important to aversive and avoidance responding than fluid availability during extinction. Because rats in Group Box only had an association between the danger context and illness, the addition of KoolAid during extinction training had no effect as there was no flavor or drinking association to extinguish. In contrast, aversive and avoidance behaviors that were established with water or a novel flavor available may display different extinction patterns when a different novel flavor is presented during extinction training. Rats in Group Box-Water and Group Box-Sacc may have had associations between the danger context and illness, with the drinking behavior

and illness, or the flavor and illness, and as a result the addition or absence of KoolAid during extinction training may influence the existing associations that do involve consumption.

General discussion

Experiment 1 showed that rats will learn to drink a novel fluid in a safe context, but not in a danger context, when the danger context is paired with illness. Differential responding also transferred to a novel flavor. In addition to differential consumption, the rats also displayed aversive behaviors in response to the danger context in a pattern that replicated previous research using this paradigm (Brown et al., 2011). In particular, gaping was significantly more frequent in the first 10 minutes of a danger trial than in the second 10 minutes, but chin rubbing was more evenly distributed throughout the entire 20-minute danger trial. The pattern of gaping over the 20-minute trial did not change when the saccharin solution was presented at the beginning of the trial or when it was never presented. However, chin rubbing responding was higher when the saccharin solution was never presented during the 20-minute trial. The differences between the patterns of gaping and chin rubbing and the effect of manipulating the presentation of the saccharin solution on chin rubbing, but not gaping, suggest that these behaviors may be measuring different associations or processes. Differences between gaping and chin rubbing is a trend that was also observed in Experiment 2, where differential chin rubbing was acquired later and extinguished sooner than differential gaping, further suggesting that these behaviors may be measuring different aspects of the context and taste aversions. These differences in aversive responding are in line with previous research by

Parker and MacLeod (1991) that demonstrated attenuation of chin rubbing, but not gaping, when an antiemetic drug was introduced.

After the initial acquisition training, transfer test, and test cycles, rats in Experiment 1 underwent 10 days of extinction trials. Following 10 days of unreinforced exposure to either the danger context alone or to the danger context and water, all rats exhibited significantly reduced gaping and increased consumption in the danger context and complete extinction of chin rubbing. There were no effects of extinction condition. Overall, the results of Experiment 1 suggest that contextual control over aversive measures (such as gaping and chin rubbing) extinguish more rapidly than avoidance measures (such as fluid consumption).

Experiment 2 investigated the roles of the context, the drinking behavior, and a novel flavor on the acquisition and extinction of differential consumption and aversive responding. While there were no differences between Groups Box, Box-Water, or Box-Sacc during acquisition, training condition did influence rats' behaviors in several measures during and after extinction. It is possible that these group differences were present during acquisition as well but that they were masked by floor effects. Support for this idea comes from the observation that differences were observed after extinction. In particular, the flavor avoidance in rats in Group Box was extinguished faster than in rats in Groups Box-Water or Box-Sacc. Similarly, the context avoidance, as measured by the PCT, was weakest in rats in Group Box-Sacc, and the flavor avoidance was slower to extinguish in rats in this group, especially when KoolAid was available during extinction training. Generally, then, the association between the context and illness was strongest in

rats in Group Box and weakest in rats in Group Box-Sacc, while the flavor avoidance was strongest in rats in Group Box-Sacc and weakest in Group Box. Responding by rats in Group Box-Water revealed a mixture of characteristics of responding by the other two groups, in that they had a context aversion similar to rats in Group Box, showed extinction of the flavor avoidance similar to rats in Group Box-Sacc, were influenced by the presence or absence of KoolAid during extinction training, and tended to gape more frequently than Group Box-Sacc.

One of the goals of Experiment 1 was to replicate and begin to elucidate the findings of Brown et al. (2011), specifically why gaping decreased in frequency after only 10 minutes of a 20-minute danger trial while chin rubbing did not. The possible reasons for these results included response fatigue, within-trial extinction, the anticipatory nausea phase evoking gaping, and a stronger context-LiCl association. While the test trials were intended to establish which mechanisms were at work with this decreased responding over time by completely overlapping the context and flavor cues and then completely separating them, the results did not lead to a clear explanation. Instead, the same pattern of gaping was established whether the rats were exposed to the danger context and saccharin solution for the full 20 minutes or to only the danger context for the entire trial. If the presence of the saccharin flavor was influencing gaping, higher gaping at the start of the complete overlap test trial would have been expected regardless of response fatigue occurring later in the trial. Alternatively, if gaping were a result of anticipatory nausea, gaping would have been less frequent when the anticipatory phase is eliminated. Instead, gaping did not differ between the two tests, suggesting that

the results observed in the distribution of gaping were not a result of saccharin solution presentation or anticipatory nausea. Comparatively, the pattern of chin rubbing did not vary between the two test trials, although the rats chin rubbed more when saccharin solution was not presented.

Because Experiment 1 left more questions about the role of the associations between the context and illness, the drinking behavior and illness, and the novel flavor and illness, Experiment 2 aimed to manipulate those associations to clarify what mechanisms are affecting the gaping, chin rubbing, and consumption results observed. When rats in Experiment 2 were trained with water or saccharin solution available the context-illness association was weaker, as measured by the PCT, while the fluid-illness association was stronger, as measured by the effects of KoolAid during extinction training. When rats were trained with only the context, the context-illness association was similar to that seen in Groups Box-Water and Box-Sacc but the fluid-illness association was weak.

One of the main goals of both Experiment 1 and Experiment 2 was to determine the underlying mechanism for the type of associative learning that takes place within this paradigm. Two of the strongest explanations for the results observed previously (e.g., Brown et al., 2011; Murphy & Skinner, 2005) are Pavlovian conditioning, where there is an association formed between the danger context and the LiCl, and occasion setting, where there is an association between the flavor and the LiCl that is modulated by the context, allowing the context to determine the “safeness” of the saccharin flavor. The success of each of these explanations can be considered by examining the strengths of the

associations between the context, saccharin flavor, and water with LiCl using the aversive and avoidance measures observed in both experiments. This has been extensively discussed in previous research (Brown et al., 2011; Murphy & Skinner, 2005; Skinner et al., 1994) and was the primary focus of a study by Iguchi et al. (2014) who suggested using the extended discrimination training and context extinction methods used in Experiment 2 to further elucidate their findings.

A Pavlovian conditioning explanation would suggest that rats in the training groups that produced the strongest context-LiCl association would also exhibit the most aversive behaviors, the strongest flavor and context avoidance, and the strongest transfer of these measures to a novel flavor. While Experiment 2 did not show any group differences after acquisition, the rate at which the measures extinguished does offer some insight into the underlying mechanisms at work. Rats in Group Box displayed the fastest extinction, and therefore the weakest association, of the flavor avoidance and aversive behaviors. Rats in Group Box-Water were also faster than rats in Group Box-Sacc to extinguish the flavor avoidance, but took longer than rats in Group Box to extinguish differential responding of gaping and chin rubbing, suggesting a slightly stronger context-LiCl association. Rats in Group Box-Sacc showed less extinction of the flavor avoidance than did rats in Groups Box or Box-Water, and extinguished differential responding of gaping and chin rubbing at the same rate as rats in Group Box-Water, suggesting the strongest context-LiCl association. However, the results of the PCT suggest the opposite strengths of association; rats in Group Box and Group Box-Water showed similar levels of context avoidance both after acquisition training and during extinction trials, while rats

in Group Box-Sacc showed no context avoidance even following acquisition. By some measures, the context-LiCl association was strongest in rats in Group Box-Sacc, but by other measures this group showed the weakest context-LiCl association. The rats in Group Box-Sacc, which had the strongest association based on the consumption and aversive responding results, had no context avoidance when this was assessed independently, suggesting that these results may be better explained by the occasion setting model whereby the context is modulating the safeness of the saccharin flavor.

The second experiment was designed to examine the associations that underlie contextual control of fluid consumption. A myriad of associations could underlie the experimental outcome observed. Neither the occasion setting nor the Pavlovian explanations readily capture the pattern of findings observed. According to the occasion setting account rats in Group Box-Sacc would have learned an association between the saccharin solution and LiCl that depended on the presence of the box color. The fact that this group had the strongest transfer suggests that conditional control of a particular flavor had not occurred. The simplest association would be a Pavlovian association between the box and LiCl. One could argue that if this was the sole association Group Box would show the strongest transfer and strongest place avoidance. Comparison of the three groups was consistent with a Pavlovian account to the extent that place avoidance was greatest in rats in Group Box, followed by rats in Group Box-Water, followed by rats in Group Box-Sacc. The weaker transfer during extinction by rat in Groups Box suggests that the Pavlovian association between the box and LiCl did not capture all the associations that occurred. The increased consumption during extinction by Group Box

relative to the other two groups suggests that conditional control of drinking was also learned during discrimination training in Groups Box-Water and Box-Sacc and that this unwillingness to drink in the presence of the box could account for the differences between these two groups and Group Box. The subsequent lack of differential consumption during the retention tests with vinegar in rats in Group Box suggests that an association between the box and a novel flavour during training was also important and this may explain why rats in Group Box-Sacc maintained differential consumption for longer than rats in Group Box-Water.

Rats in Group Box had fewer aversive responses following extinction than rats in Groups Box-Water and Box-Sacc, which did not differ. This observation suggests that in addition to a Box-Licl association there was a Box-Fluid-LiCl association that underlay the conditioned aversive responses. This drinking response association may explain why Group Box-Water performed similarly to Group Box in some measures, but similarly to Group Box-Sacc in others.

While no single explanation can account for all the findings observed in these experiments, further research into the nature of the associations formed between a flavor, context, and illness will help elucidate the mechanisms at work and how they interact with each other. Additionally, further research using several measures of avoidance and aversion, rather than only consumption, can help to explain the discrepancies within the taste learning literature. It is difficult to measure taste-potentiated context aversion while measuring only consumption, an avoidance behavior. Finally, using appetitive measures,

such as tongue protrusions, along with avoidance and aversive measures may help further clarify the associations formed by animals in flavor discrimination paradigms.

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Table 1

Outline of procedures in Experiment 2

Group	Discrimination training	Place choice*	Transfer test		Extinction					
			Test 1	Retention*	Trials 1-10	Place choice	Retention	Trials 11-19	Place choice	Retention
Group Box	Safe – Sal Danger – LiCl Safe – Sal	Choice between safe and danger context	Safe – Vin Danger - Vin	Safe Danger	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Vin Danger - Vin	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Vin Danger - Vin
Group Box-Water	Safe – Water – Sal Danger – Water – LiCl Safe – Water - Sal	Choice between safe and danger context	Safe – Vin Danger - Vin	Safe – Water Danger – Water	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Water Danger - Water	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Water Danger - Water
Group Box-Sacc	Safe – Sacc – Sal Danger – Sacc – LiCl Safe – Sacc – Sal	Choice between safe and danger context	Safe – Vin Danger - Vin	Safe – Sacc Danger - Sacc	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Sacc Danger - Sacc	½ Danger ½ Danger - KoolAid	Choice between safe and danger context	Safe – Sacc Danger - Sacc

Note: Half of the rats in each group received the black box for the danger context and the white box for the safe context, while the other half received the opposite contingency. The order of safe and danger context presentation was counterbalanced for transfer and retention tests. *A 3-day discrimination retraining cycle was given after the first place choice test and before the start of extinction trials.

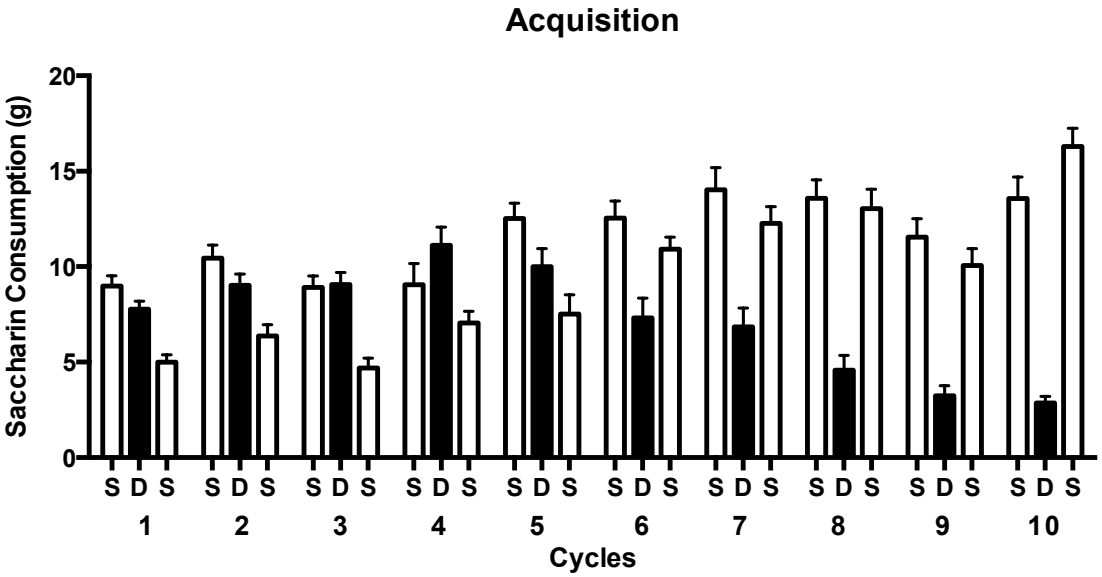

Figure 1. Mean (+ SEM) amount of a 0.1% saccharin solution consumed (g) on each safe-danger-safe (S-D-S) cycle of discrimination training (Cycles 1 through 10) of Experiment 1.

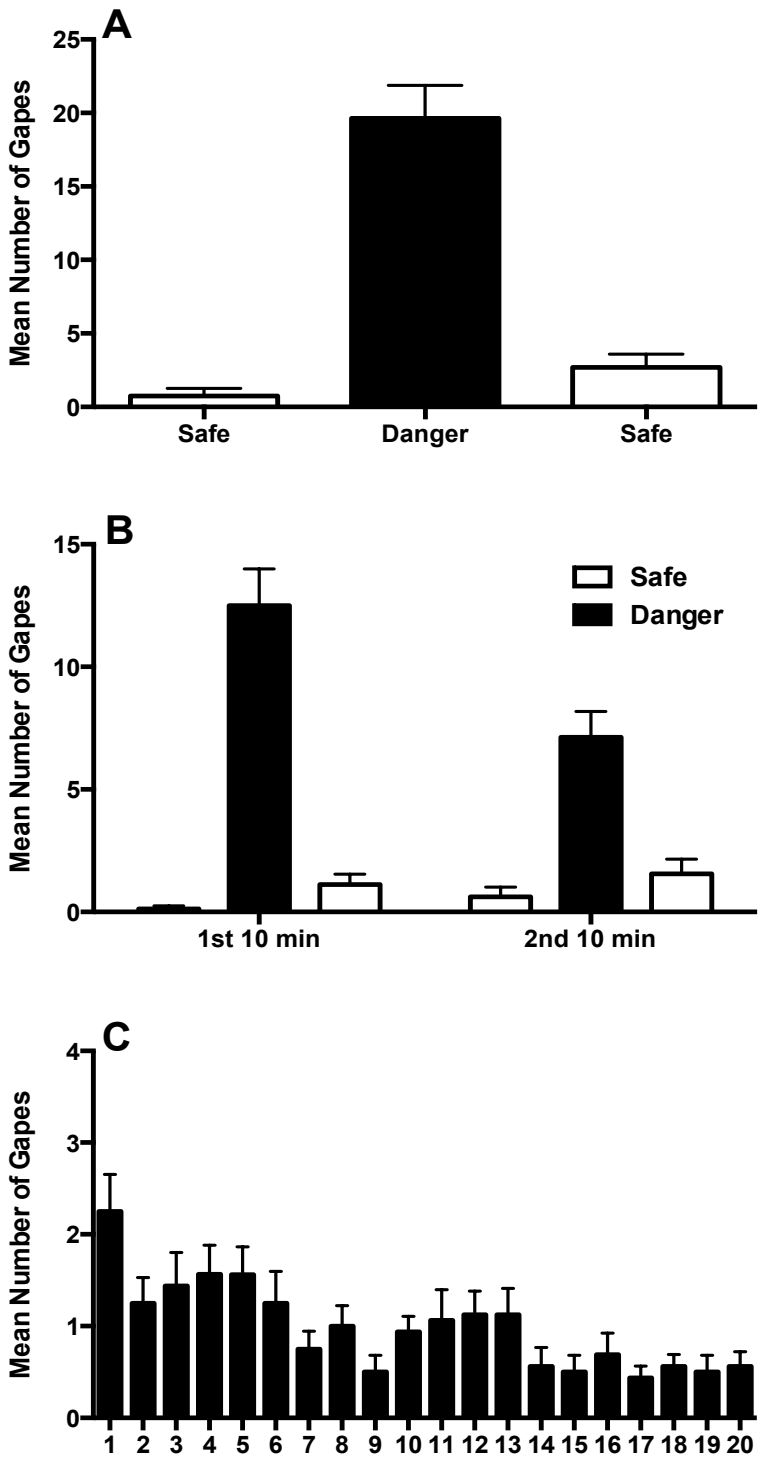

Figure 2. Mean (+SEM) number of gapes over each safe-danger-safe day of cycle 10 of discrimination training (A), for each 10-min half of each day of cycle 10 (B), and each minute of the danger trial of cycle 10 (C).

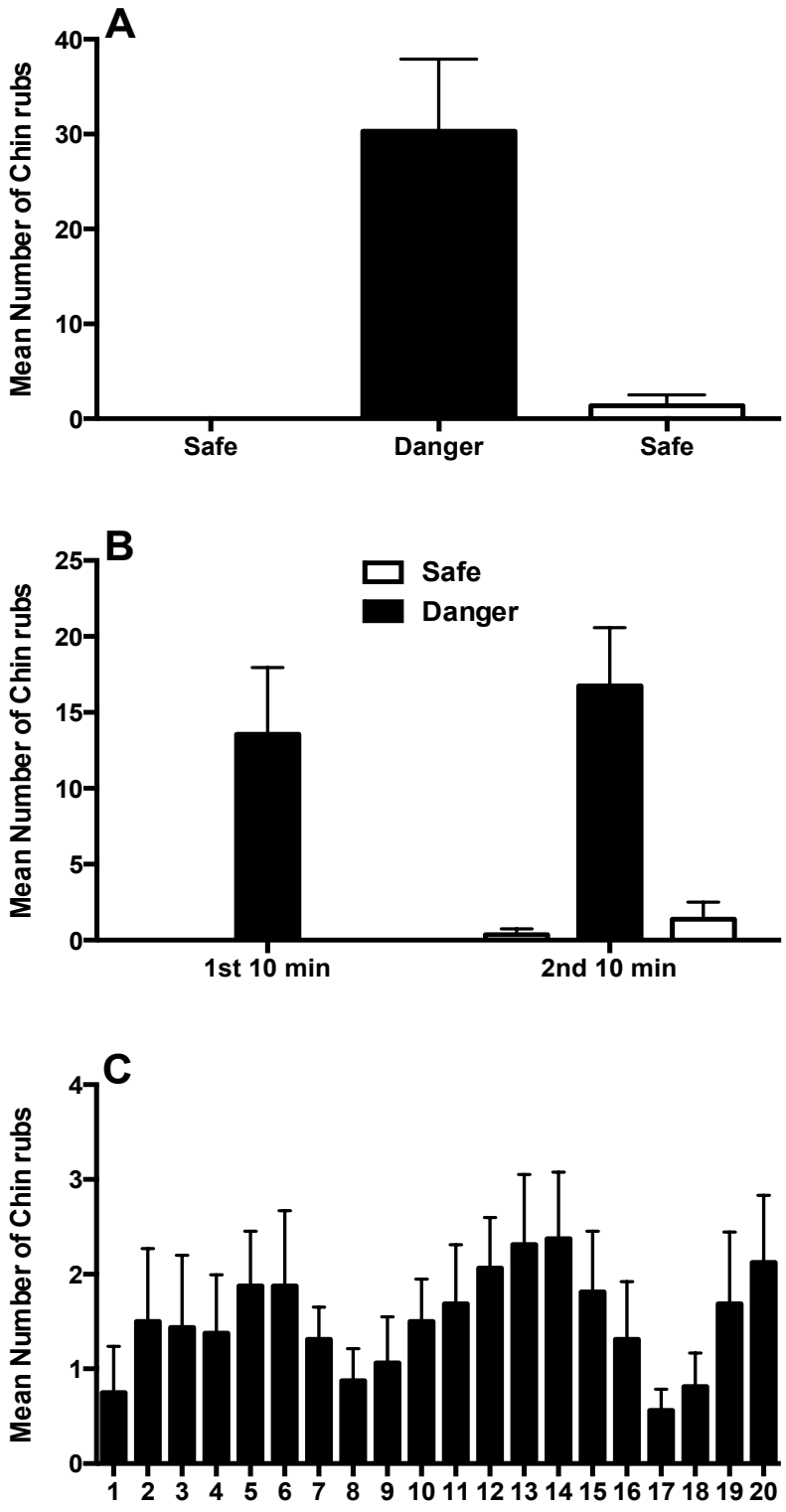

Figure 3. Mean (+SEM) number of chin rubs over each safe-danger-safe day of cycle 10 of discrimination training (A), for each 10-min half of each day of cycle 10 (B) and each minute of the danger trial of cycle 10 (C).

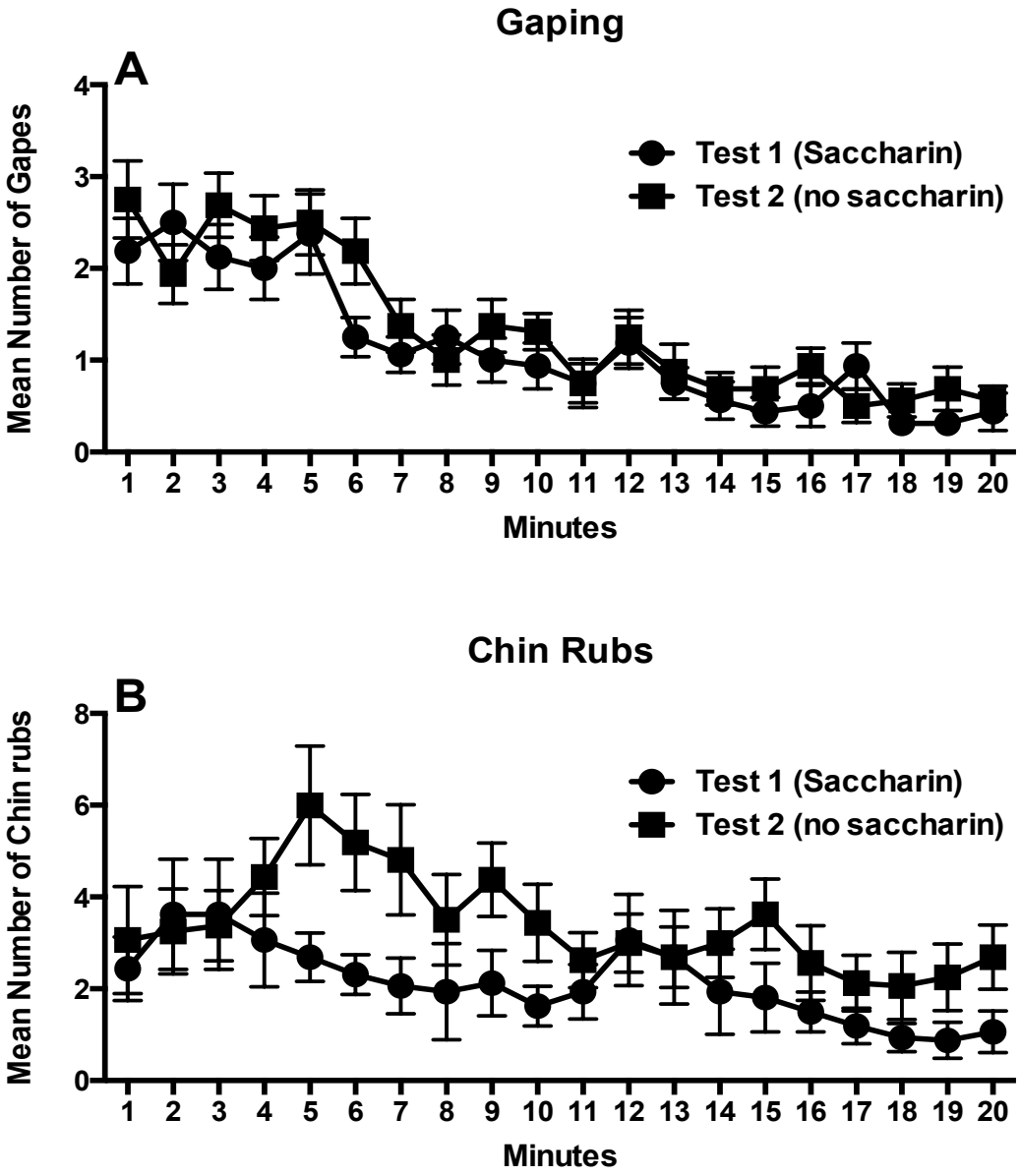

Figure 4. Mean (±SEM) number of gapes (A) and chin rubs (B) during each minute of the danger trial of test cycles 1 and 2, where saccharin solution was available or withheld, respectively, for the full trial.

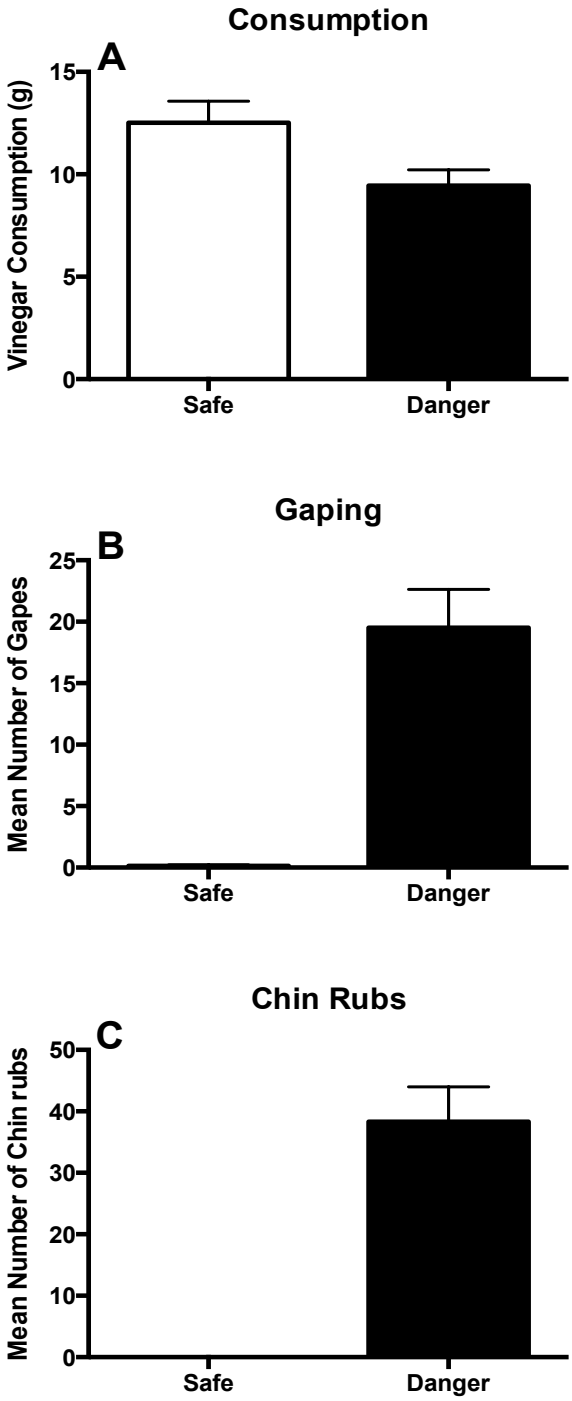

Figure 5. Mean (+SEM) amount of vinegar solution consumed (g; top panel), and number of gapes (middle panel) and chin rubs (bottom panel) observed during the safe and danger trials of the transfer test.

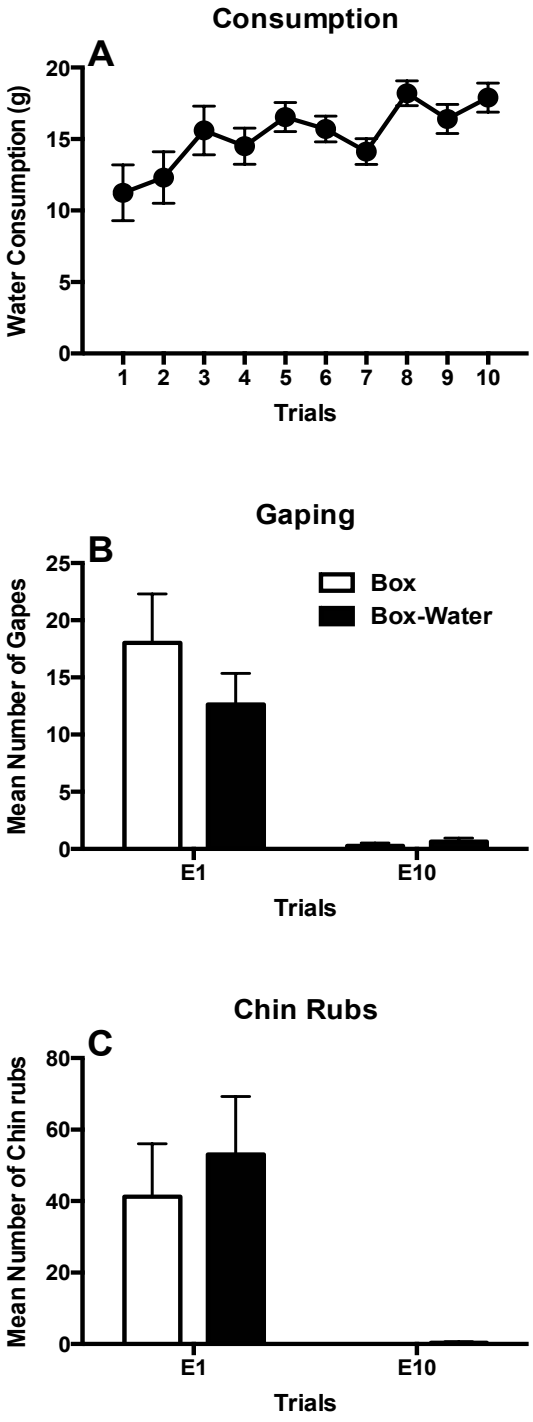

Figure 6. Mean (±SEM) amount of water consumed (g) on each extinction trial (1 through 10) for group Box-Water (A), and mean (+SEM) number of gapes (B) and chin rubs (C) on extinction trials 1 and 10.

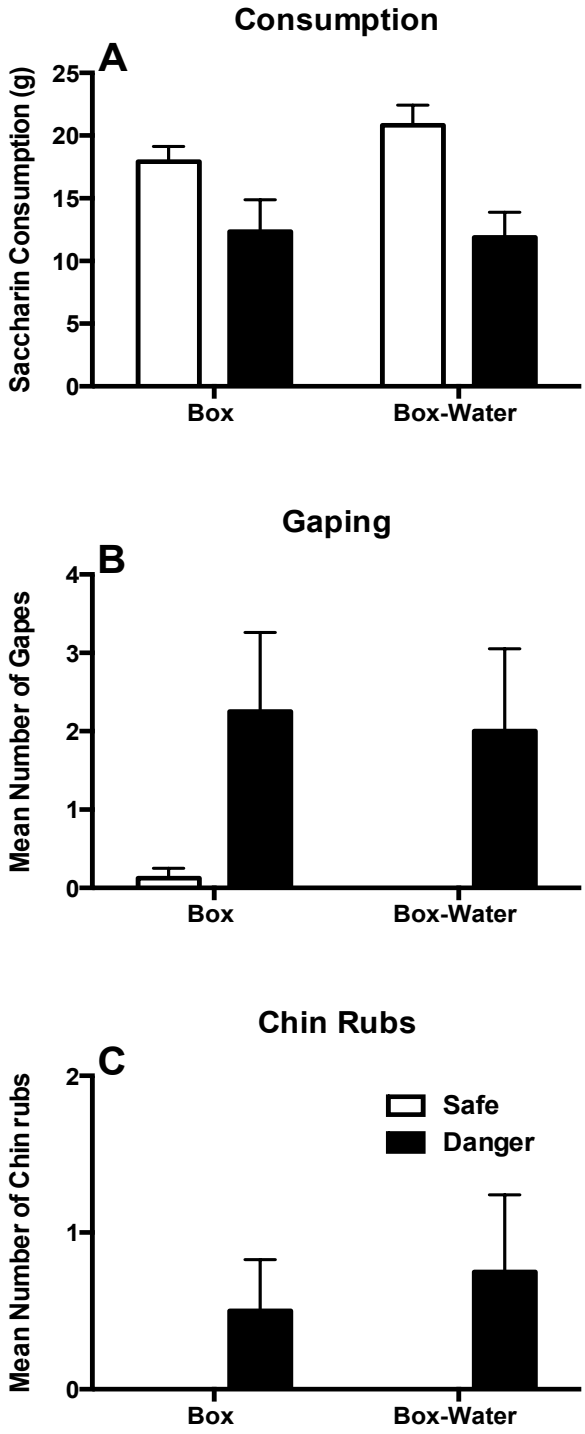

Figure 7. Mean (+SEM) amount of 0.1% saccharin solution consumed (g; A), and number of gapes (B) and chin rubs (C) made during the first safe trial (white) and the danger trial (black) of cycle 10 of discrimination training and the safe and danger trials of the retention test that followed extinction training without access to water.

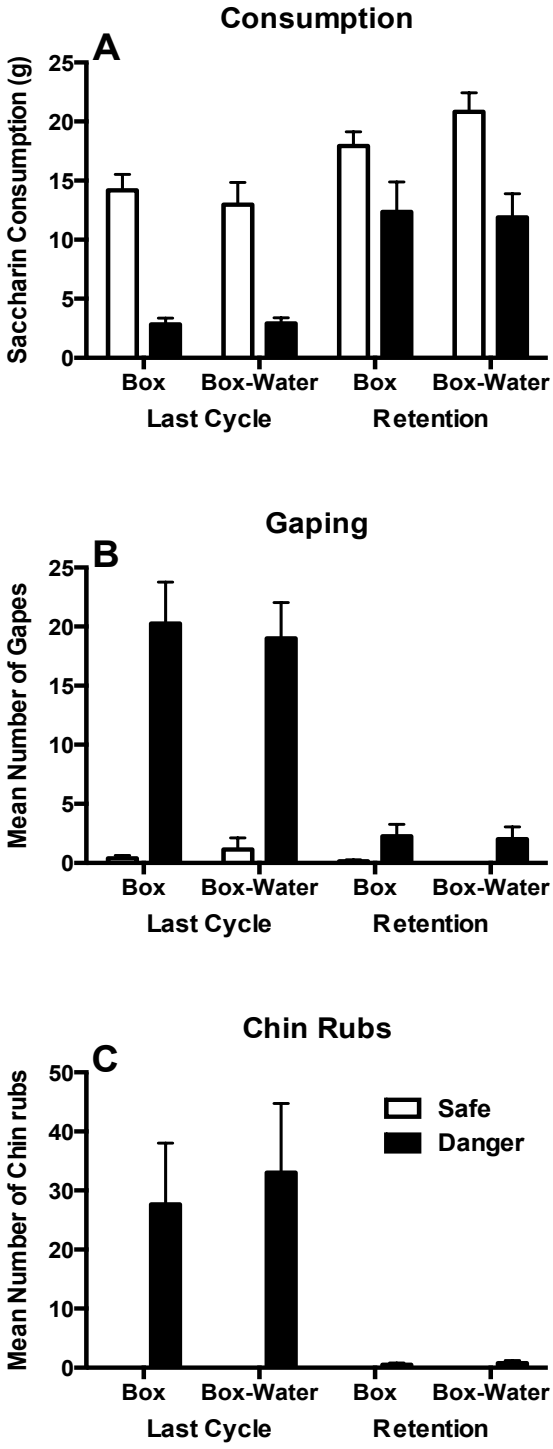

Figure 8. Mean (+SEM) amount of 0.1% saccharin solution consumed (g; A), and number of gapes (B) and chin rubs (C) made during the first safe trial (white) and the danger trial (black) of cycle 10 of discrimination training and the safe and danger trials of the retention test that followed extinction training with access to water.

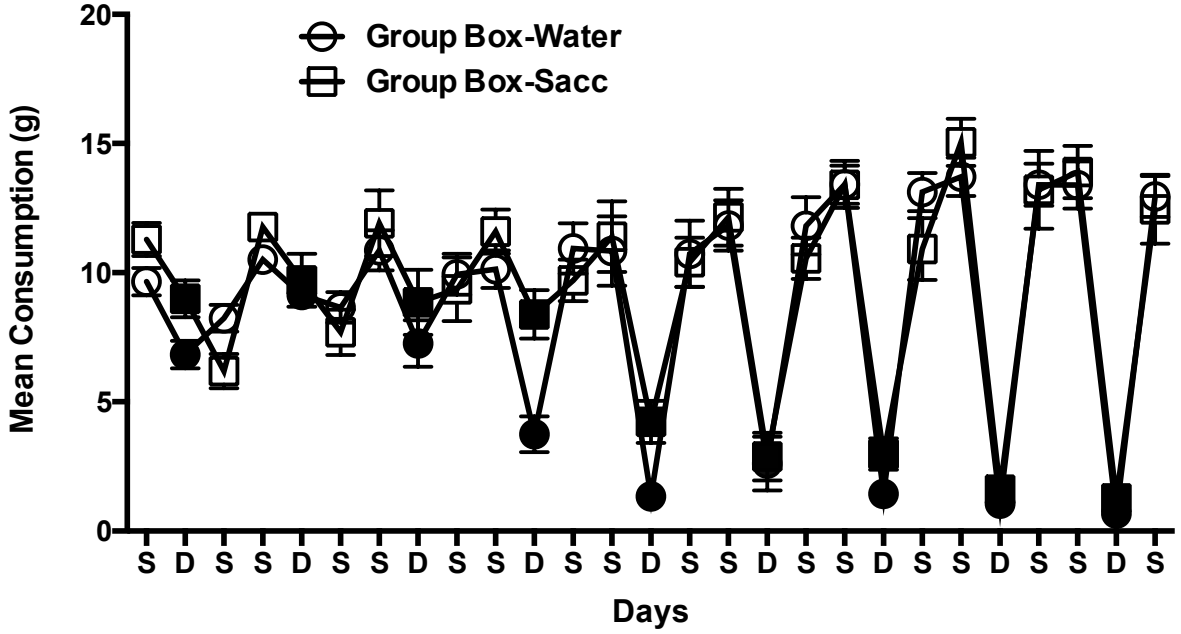

Figure 9. Mean (±SEM) amount of a 0.1% saccharin solution consumed (g) on each safe-danger-safe (S-D-S) cycle of discrimination training during Experiment 2 (cycles 1 through 9).

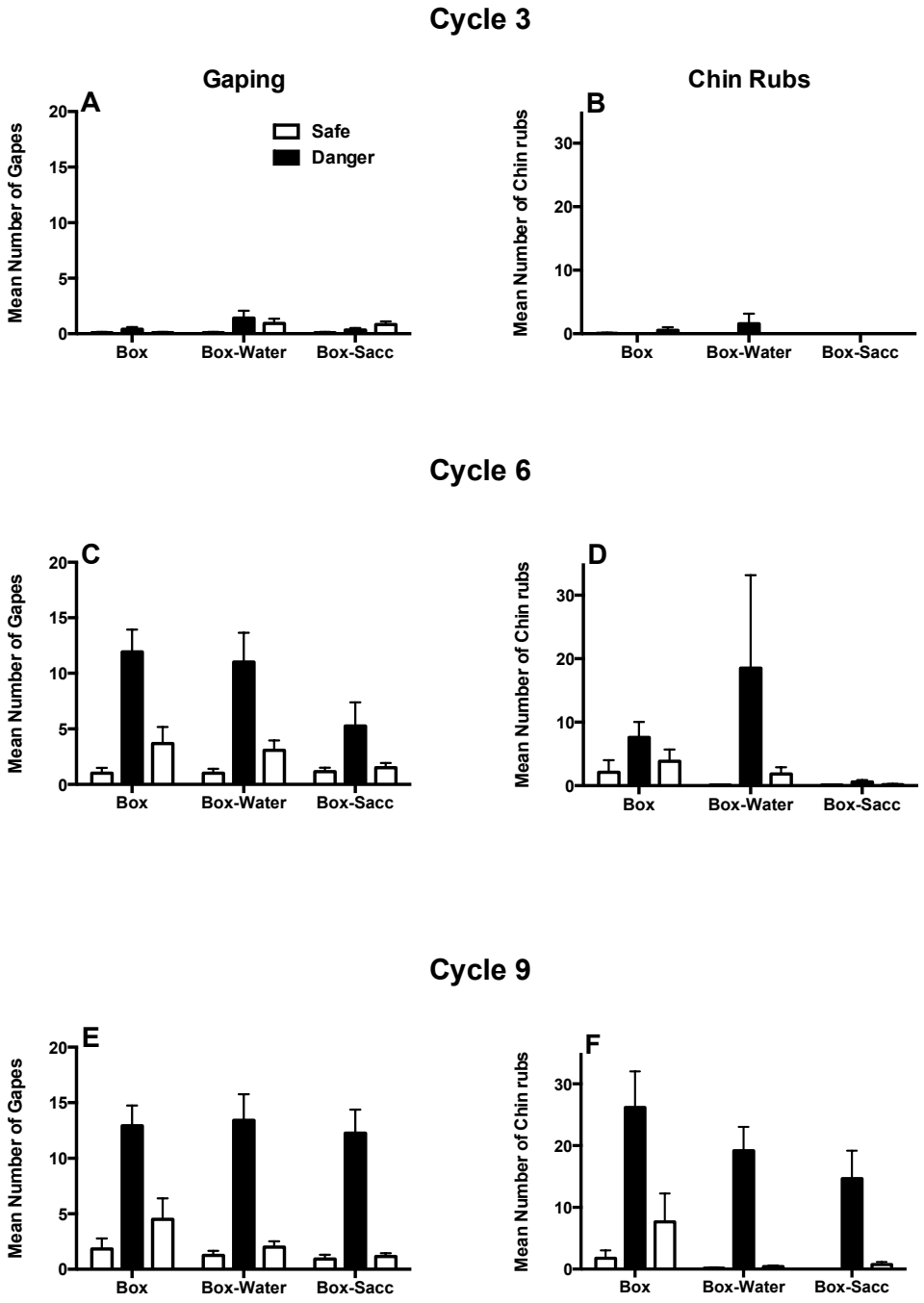

Figure 10. Mean (+SEM) number of gapes (left panel) and chin rubs (right panel) for Group Box, Group Box-Water, and Group Box-Sacc during Cycle 3 (A and B), Cycle 6 (C and D) and Cycle 9 (E and F) of discrimination training.

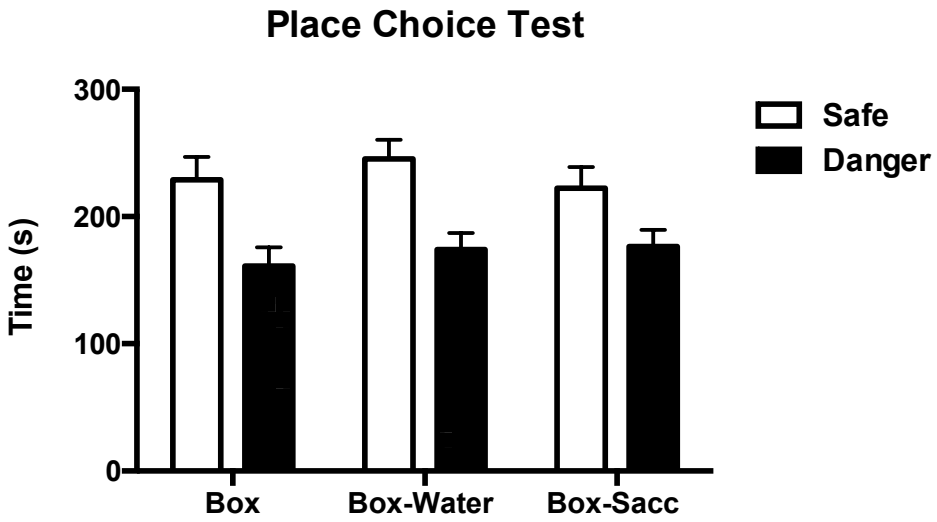

Figure 11. Mean time (s; +SEM) spent in the safe and danger contexts during the first Place Choice Test by rats in Group Box, Group Box-Water, and Group Box-Sacc.

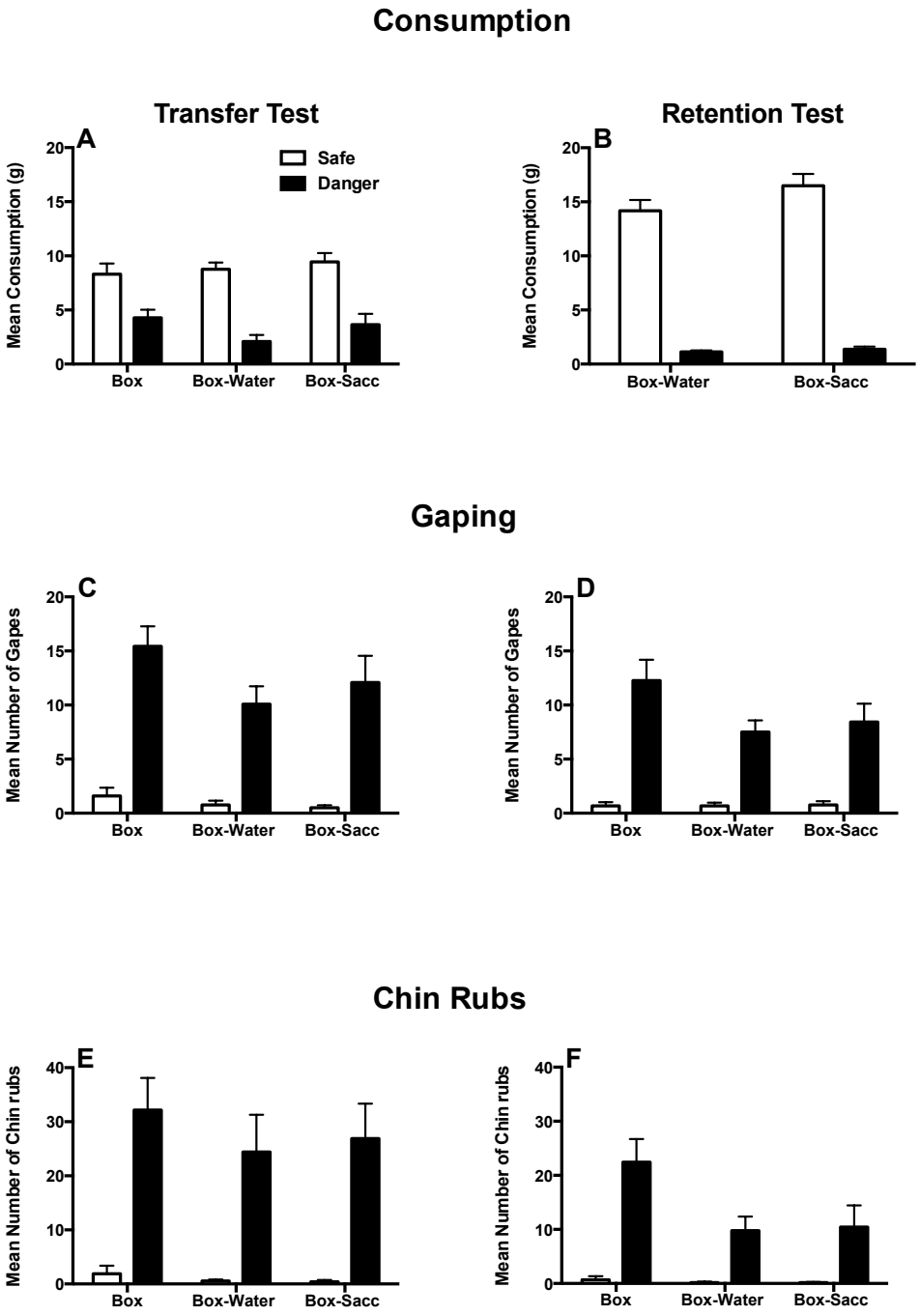

Figure 12. Mean consumption (g; +SEM) of a novel 2% white vinegar solution during the transfer test (A) and the training fluid during the first retention test (B), followed by the mean number (+SEM) of gapes (C and D) and chin rubs (E and F) during the same tests, by rats in Group Box, Group Box-Water, and Group Box-Sacc.

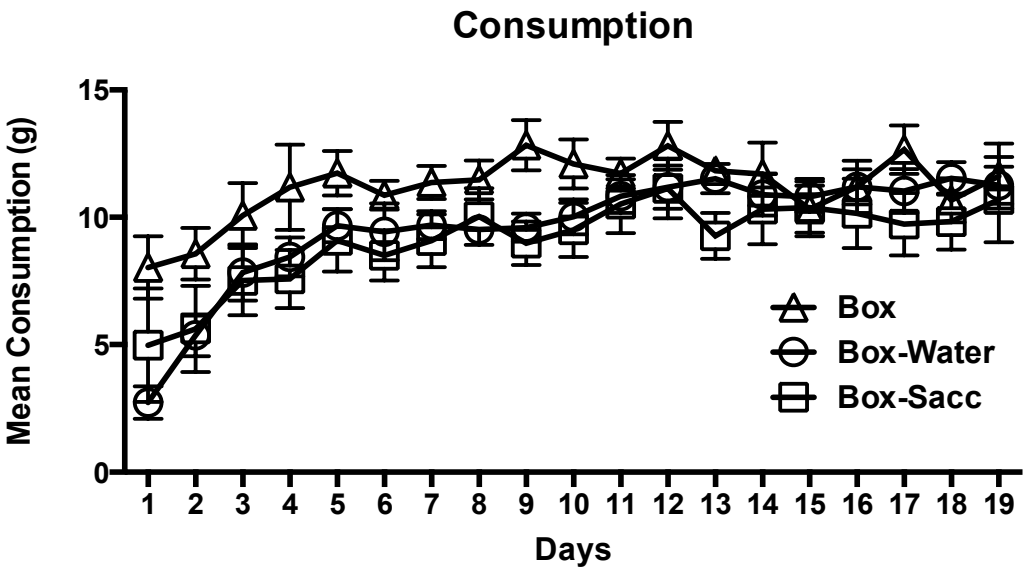

Figure 13. Mean (±SEM) amount of unsweetened cherry KoolAid (g) consumed during extinction trials (1 through 19) by rats in Group Box-FE, Group Box-Water-FE, and Group Box-Sacc-FE.

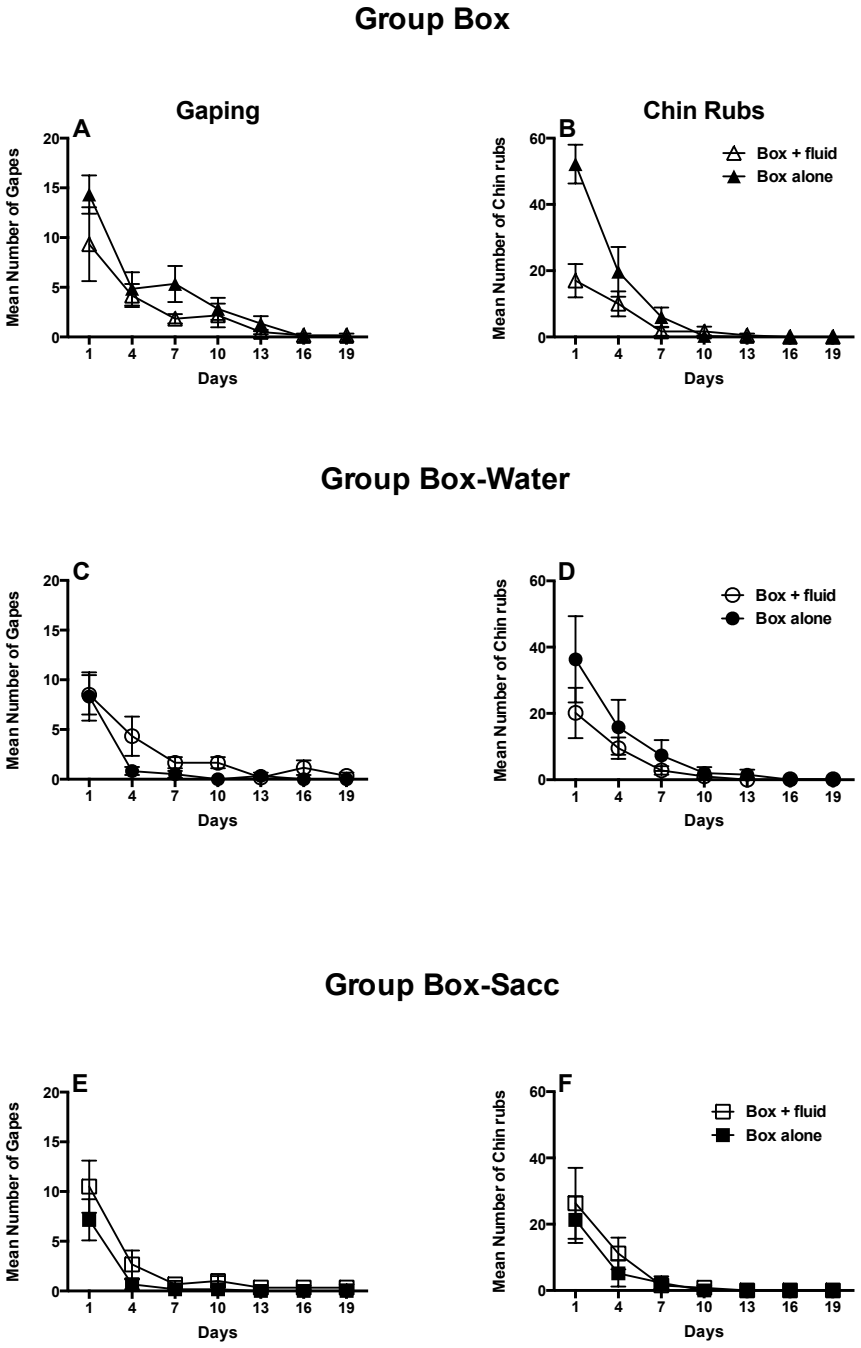

Figure 14. Mean (±SEM) number of gapes (left panel) and chin rubs (right panel) during extinction trials (1, 4, 7, 10, 13, 16, and 19) by rats in Group Box-FE and –NFE (A and B), Group Box-Water-FE and –NFE (C and D), and Group Box-Sacc-FE and –NFE (E and F).

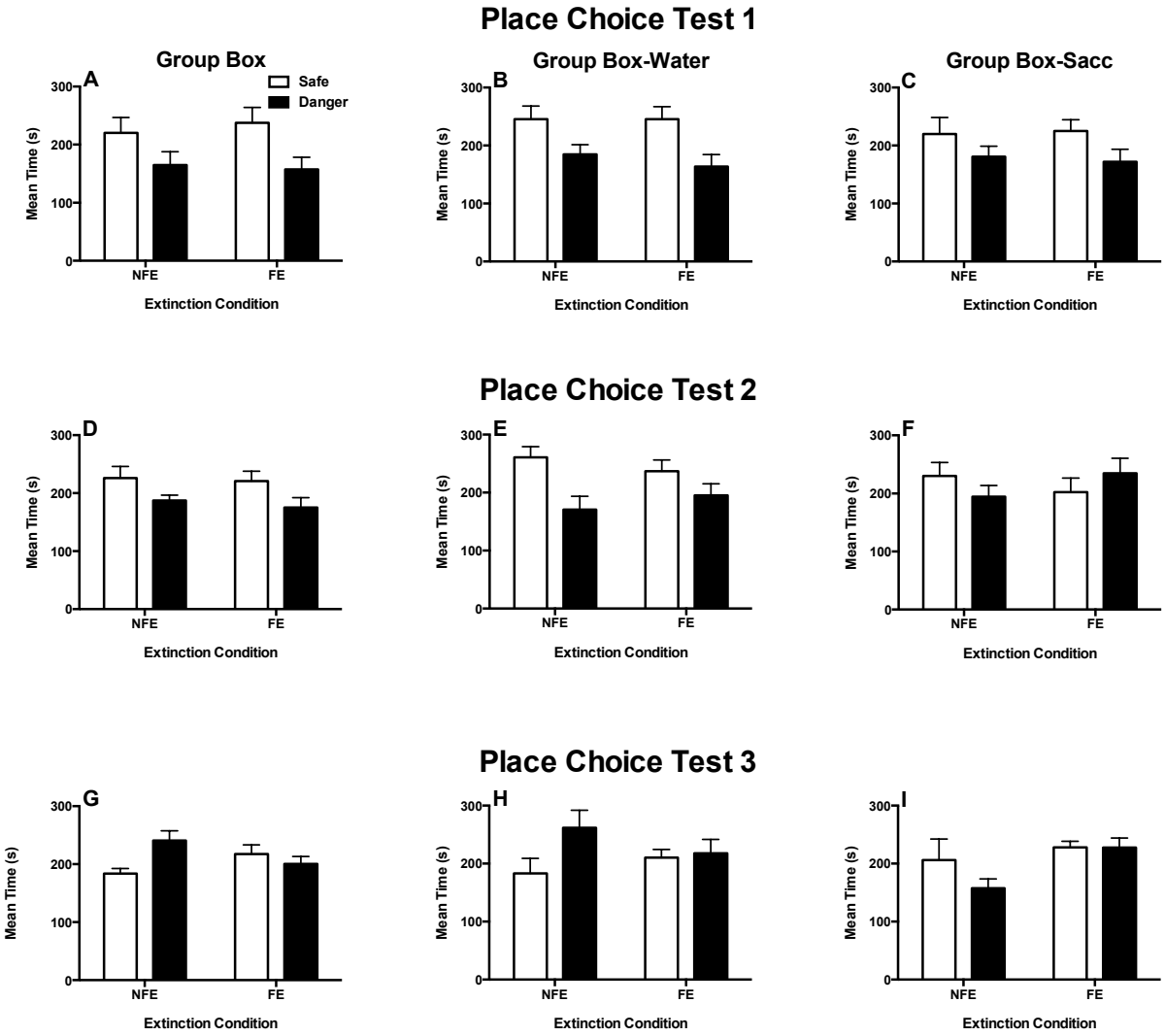

Figure 15. Mean (+SEM) time (s) spent in the safe and danger contexts during Place Choice Test 1 (top row), 2 (middle row), and 3 (bottom row) by rats in Group Box-FE and -NFE (A, D, and G), Group Box-Water-FE and –NFE (B, E, and H), and Group Box-Sacc-FE and –NFE (C, F, and I).

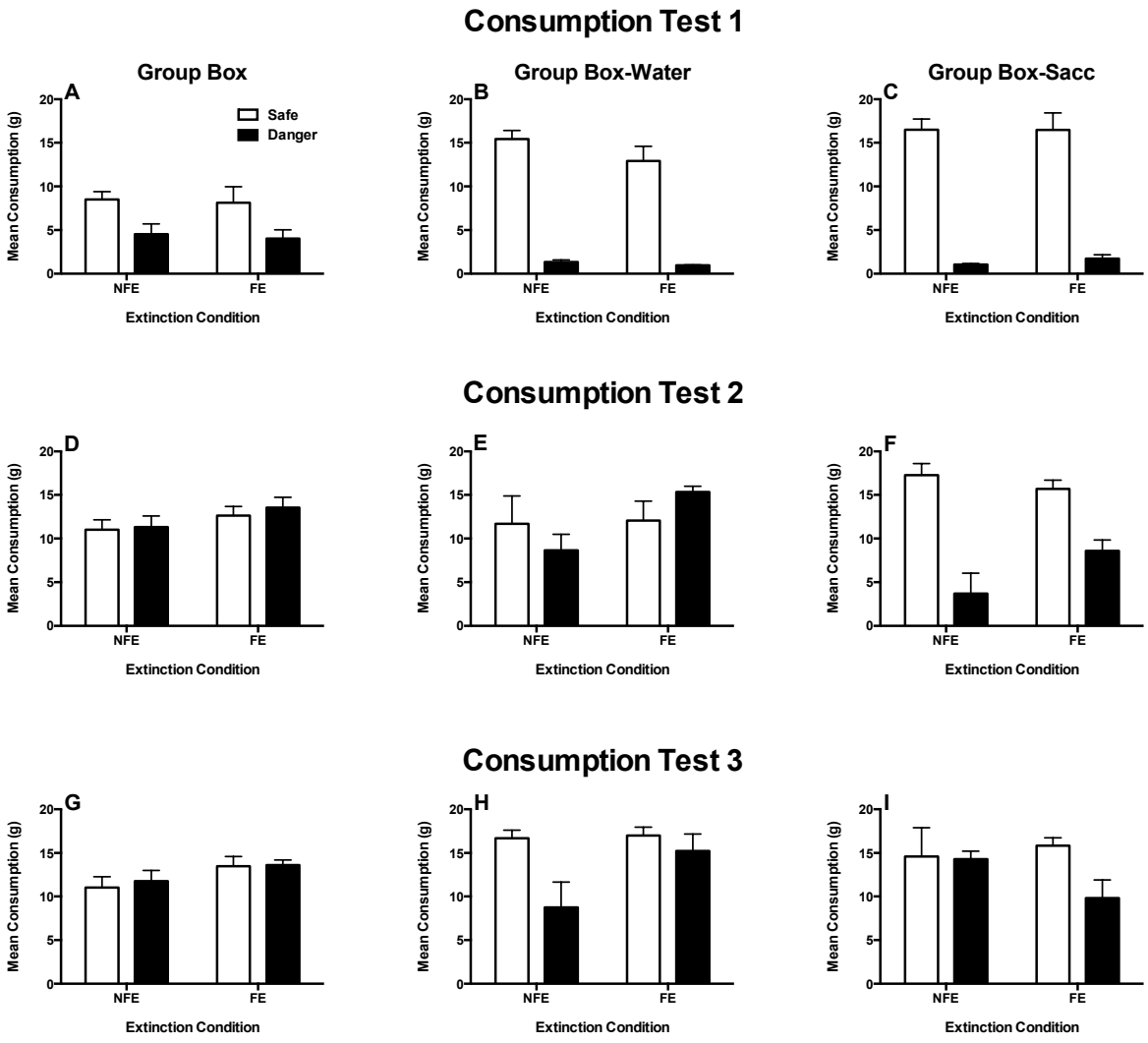

Figure 16. Mean (+SEM) amount of 2% vinegar solution, water, and 0.1% saccharin solution (g) consumed by rats in Group Box-FE and –NFE (A, D, and G), Group Box-Water-FE and –NFE (B, E, and H), and Group Box-Sacc-FE and –NFE (C, F, and I), respectively, during Consumption Tests 1 (top row), 2 (middle row), and 3 (bottom row).

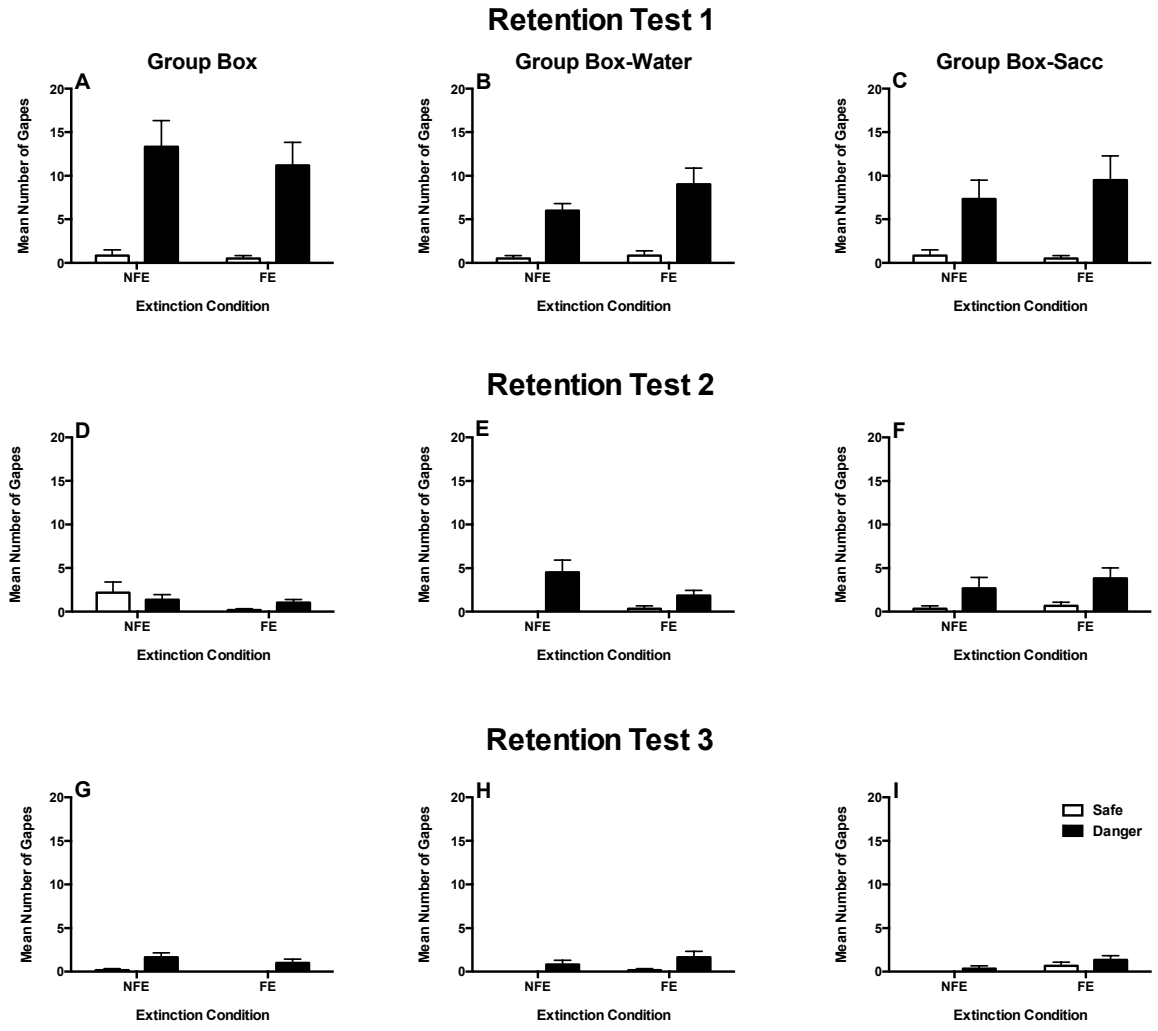

Figure 17. Mean (+SEM) number of gapes during Retention Test 1 (top row), 2 (middle row), and 3 (bottom row) by rats in Group Box-FE and –NFE (A, D, and G), Group Box-Water-FE and –NFE (B, E, and H), and Group Box-Sacc-FE and –NFE (C, F, and I).

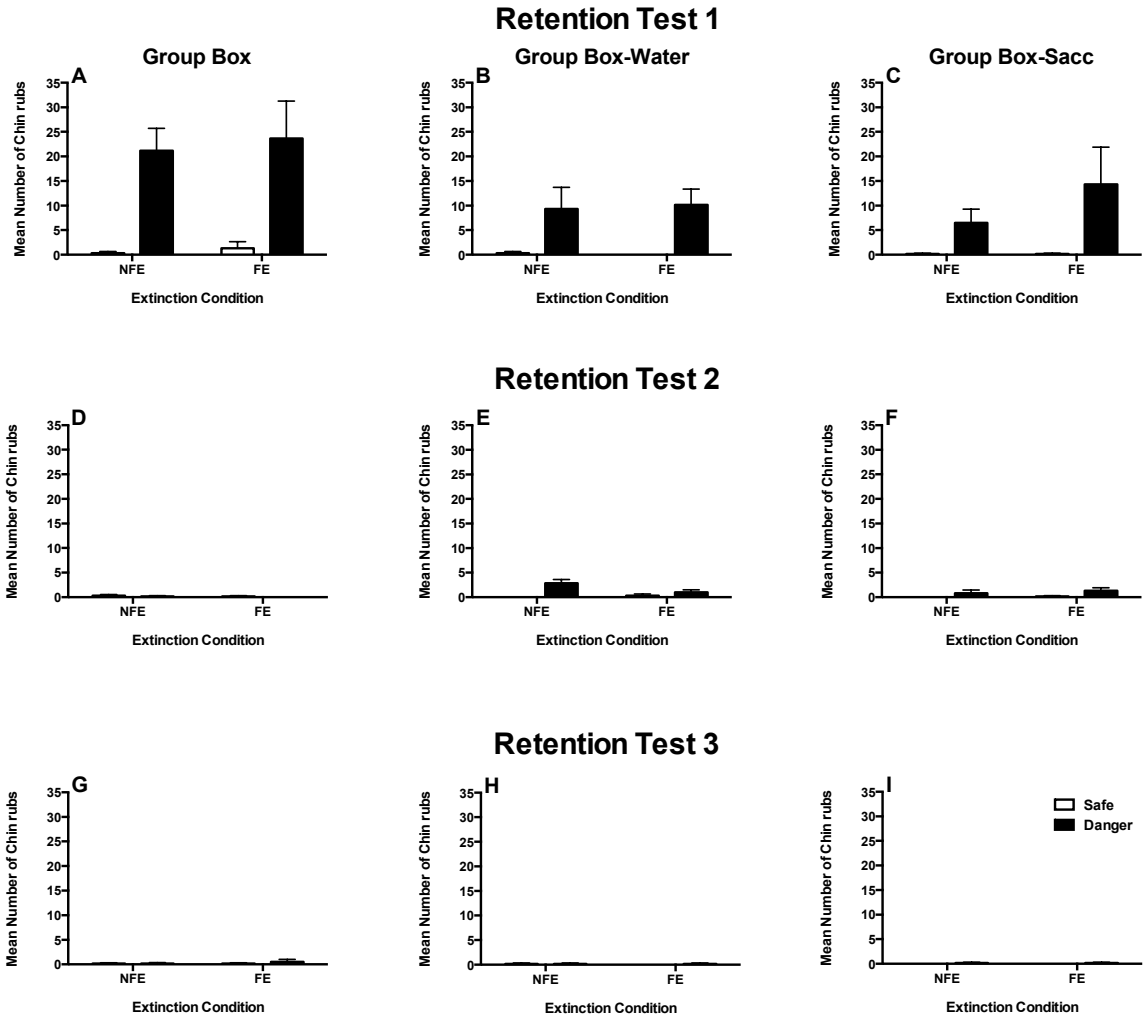

Figure 18. Mean (+SEM) number of chin rubs during Retention Test 1 (top row), 2 (middle row), and 3 (bottom row) by rats in Group Box-FE and –NFE (A, D, and G), Group Box-Water-FE and –NFE (B, E, and H), and Group Box-Sacc-FE and –NFE (C, F, and I).