Blocking of flavor-nausea learning by non-flavor cues:

Assessment through orofacial reactivity responses

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Abstract

We investigated, using orofacial reactivity assessment, whether non-flavor context cues can elicit conditioned aversive reactions, and also whether context cues interfere, through blocking, with the reduction in taste palatability during taste aversion conditioning. Experiment 1 showed that a context previously paired with LiCl evoked aversive orofacial reactions, and also attenuated the reduction in palatability of a saccharin solution which was paired with LiCl in that context. In Experiment 2, this blocking effect was abolished when the rats were given non-reinforced exposure to the previously LiCl-paired context (context extinction) before aversive conditioning of the saccharin in compound with the context. These results confirm that context stimuli can elicit conditioned aversive reactions in the absence of any flavor component, and demonstrate that context cues can interfere with the affective aspects of taste aversion learning. Thus non-flavor cues appear to engage the same processes as taste cues in aversion learning. These results are consistent with the idea that taste aversion learning is governed by general associative mechanisms and the special properties of nausea, rather than by a selective mechanism for poison-avoidance.

Keywords: conditioned taste aversion, taste palatability, context blocking, taste reactivity, rats

Rats readily learn to avoid foods paired with toxins that have previously caused them gastrointestinal malaise (Garcia, Hankins, & Rusiniak, 1974). This phenomenon is termed conditioned taste aversion (CTA) and potentially represents a key behavioral mechanism for toxin avoidance (Domjan, 1980; Garcia, Kimmeldorf, & Koelling, 1955; Garcia & Koelling, 1967; Reilly & Schachtman, 2009). Moreover, pairing a novel taste with nausea produced by a drug with emetic properties (such as lithium chloride; LiCl) not only results in a reduction in consumption of that taste, but also produces a reduction in its affective value or palatability that can be revealed through a range of techniques (for reviews see, Lin, Arthurs, & Reilly, 2014; Parker, Rana, & Limebeer, 2008). In addition, classic studies (e.g. Garcia & Koelling, 1966) suggest selective learning effects where rats learn more readily to associate tastes with internal aversive events (e.g. nausea) than with external aversive events (e.g. pain produced by footshock) and that the reverse is true with audiovisual stimuli. However, there is also clear evidence that pairing contextual cues with the aversive effects of LiCl will support at least some conditioned responses (e.g. Batson & Best, 1979; Boakes, Westbrook, & Barnes, 1992). Thus one key issue in the analysis of CTA is whether non-flavor stimuli engage the same range of processes as do foods and flavors in aversion learning. This is particularly important in the context of modelling anticipatory nausea in chemotherapy which can be elicited by the treatment context (Parker, 2014). Here, we address the critical question of how non-flavor cues are engaged in "taste" aversion learning by examining affective responses elicited by LiCl-paired contexts, and by examining the ability of context cues to block changes in affective responses to taste stimuli. But before turning to the analysis of contexts and aversion learning, it is important to consider the experimental methods used to assess affective responses in rodents.

In the orofacial reactivity test[1], rats are implanted with intraoral cannulas and the orofacial and somatic responses accompanying an intraoral infusion of the taste are recorded (Grill & Norgren, 1978). This supports a direct examination of the affective responses elicited by the infused solution. These can be classified as aversive (i.e., rejection responses) such as gaping, chin rubbing, and paw treading (elicited, for example, by unpleasant sour or bitter tastes), or appetitive (i.e. ingestive responses) such as tongue protrusions and paw licks (elicited, for example, by pleasant sweet tastes). In terms of taste aversion, Pelchat, Grill, Rozin, and Jacobs (1983) observed that pairing sucrose with LiCl-induced nausea reduced both voluntary consumption of sucrose and a change in orofacial reactivity responses (both an increase in aversive responses and a decrease in appetitive responses). It should be noted that appetitive and aversive orofacial responses can be interpreted as lying on either a single dimension (from highly positive with large numbers of appetitive responses to highly aversive with large numbers of aversive responses – with an intermediate point with low numbers of either appetitive or aversive responses: see for example, Breslin, Grill, & Spector, 1992); or on two separate dimensions (where aversive responses are indicative of disgust and appetitive responses are indicative of positive hedonic value – with these being at least partially independent of each other: see for example, Berridge & Grill, 1983, 1984). While these differences in interpretation are important and have a material impact on some aspects

[1] This method was originally described as the taste reactivity test. Although this terminology remains in common use (especially because it is most commonly applied to taste and flavor stimuli), we have chosen to emphasize the nature of the response here because we are considering its application to non-taste stimuli. There are also other means of assessing palatability, most notably the analysis of licking microstructure (e.g., Davis, 1989; Dwyer, 2012; Lin et al., 2014). Although this has been used extensively in the examination of taste aversion learning (e.g., Arthurs, Lin, Amodeo, & Reilly, 2012; Baird, St. John, & Nguyen, 2005; Dwyer, Gasalla, & López, 2013) we will not consider it in detail here because the method requires voluntary consumption and is thus unsuitable for examining responses to contextual stimuli.

of the analysis of taste aversion learning (compare, for example, Lin, Arthurs, & Reilly, 2016; with, Parker, 2014) they share the important common ground that one of the distinct features of taste aversion learning is a reduction in affective value and palatability. We do not take a strong position on the merits of the one- or two-dimensional interpretations of the orofacial reactivity responses here, save to note that either an increase in aversive responses or a decrease in appetitive responses would be indicative of a reduction in palatability or affective value.

Returning to the central question of how non-flavor stimuli engage in aversion learning, it is well established that pairing contextual cues with LiCl will endow the context with at least some conditioned aversive properties. However, the full nature of these aversive properties has yet to be determined. In particular, it is not clear whether context-nausea pairings endow the context with the same range of conditioned aversive properties as do taste-nausea pairings. Many different test procedures have been employed to assess context aversion learning, including the amount consumed of a palatable solution in the conditioned context (Best, Brown, & Sowell, 1984; Boakes et al., 1992), the amount of time spent in an environment previously paired with LiCl on a place-preference test (Tenk, Kavaliers, & Ossenkopp, 2005; White & Carr,1985), and blocking of a taste aversion by prior nausea-based context conditioning (Batson & Best, 1979; Willner, 1978). Using a blocking procedure, Batson and Best (1979), for example, reported that rats previously given pairings of a black chamber with LiCl-induced nausea showed reduced aversion to a saccharin solution that was presented in compound with the pretrained context and followed by LiCl administration. This result has been confirmed through the use of conceptually similar blocking procedures (Krane, 1980; Kwok & Boakes, 2012; Rodríguez, López, Symonds, &

Hall, 2000; Symonds & Hall, 1997; Symonds, Hall, López, Loy, Ramos, & Rodríguez, 1998) demonstrating that pretrained contextual cues reliably interfere with taste aversion learning[2].

At present, in all of these context-blocking studies, the degree of aversion was assessed through examining the amount of the flavor consumed at test, the most commonly employed measure of a conditioned aversion. However, as mentioned previously, taste aversion learning not only reduces voluntary consumption of the flavor, it also results in reduction in the affective value of that flavor. Most notably, normally positive flavors (such as sucrose or saccharin) paired with nausea induced by LiCl come to elicit fewer appetitive and more aversive orofacial reactivity responses than they would otherwise. Critically, the reduction in palatability depends on the nature of the aversive stimulus: in some cases, such as pain produced by footshock, a reduction in consumption is not accompanied by an increase in aversive orofacial reactions (e.g. Pelchat et al., 1983). Thus measures of voluntary consumption alone do not provide a full picture of the nature of context-nausea learning because this does not address the issue of a reduction in affective value.

Using the analysis of orofacial reactivity, there is some recent evidence showing that rats might display conditioned changes in affective value to a contextual cue paired with LiCl. The first example comes from Limebeer, Hall and Parker (2006), who reported that when rats were repeatedly infused with saccharin in a context previously paired with LiCl, they not only displayed aversive orofacial reactivity reactions during the flavor infusions but also during the inter-infusion intervals, indicating that contextual cues can acquire the ability to elicit responses indicating reduced affective value. However, in these experiments an

[2] Although blocking is commonly interpreted as a deficit in acquisition, there are demonstrations of recovery from blocking suggestive of performance/retrieval effects (Blaisdell, Gunther, & Miller, 1999; Pineno, Urushihara, & Miller, 2005). None of the studies of context blocking of taste aversion noted here distinguish between contributions of acquisition or performance to the observed effects.

explicit olfactory cue (vanilla flavor extract) was used as a part of the context. Thus the aversive orofacial reactivity reactions to the context may have actually been elicited only by this flavor component. Therefore, examining contexts without an odor component is necessary for a true comparison between flavor and non-flavor stimuli. Two previous studies examined this issue. In an experiment reported by Limebeer, Krohn, Croos-Mellor, Litt, Ossenkopp, and Parker (2008), rats displayed conditioned aversive orofacial reactivity reactions during exposure to a distinctive context paired with LiCl even in the absence of a flavored solution or any odor cue. However, in a similar experiment by Meachum and Bernstein (1992) animals displayed conditioned aversive orofacial reactivity responses to a context paired with LiCl only if an odor cue (pine scent) was presented as a part of the context. Therefore, the ability of non-flavor context cues to elicit conditioned aversive orofacial reactivity responses remains controversial.

Whereas it has long been known that nausea-based context conditioning can interfere with taste aversion as revealed by suppressed consumption, its effectiveness in blocking changes in affective value has not been yet examined. It is therefore unclear if context blocking of a taste aversion reflects a modulation of affective value (such that the reduction in taste palatability is attenuated as a result of context blocking) or is simply the result of reduced avoidance independent of changes in affective value. In short, it is not yet clear whether context-nausea pairings result in the context acquiring the same conditioned properties as do nausea-paired taste stimuli. Therefore, Experiment 1 tested context-blocking of affective value by examining orofacial reactivity responses elicited by the context cues, and by saccharin that had been trained in a context which had, or had not, been previously paired with LiCl. Experiment 2 extended this to examine whether blocking of conditioned changes in affective reactions to saccharin was obtained when rats were given non-reinforced exposure to the context previously paired with the LiCl (i.e., context extinction) before the

saccharin was conditioned in compound with the context. Both experiments also examined the ability of contextual cues paired with LiCl to directly elicit orofacial responses indicative of changes in affective value.

Experiment 1

The specific aim of this experiment was to test whether a context previously paired with lithium injections interferes with conditioned changes in the palatability of a LiCl-paired flavor as assessed by the orofacial reactivity test. In a pilot study one group of rats was injected with LiCl (pretrained group) and the other with saline (non pretrained) before being placed in a distinctive context produced by the chamber used for intra-oral (IO) infusion and recording of orofacial reactions. All rats subsequently received an IO infusion of saccharin while in the conditioning chamber followed by an injection of LiCl. During test, rats were IO infused with the saccharin and the pretrained group displayed fewer aversive orofacial reactions than the non pretrained group, suggesting that pretraining with LiCl interfered with the reduction in saccharin palatability. These results are consistent with the idea that a LiCl-paired context can block the conditioned changes in the affective value of a flavor. However, the blocked cue (saccharin) was presented in compound with the blocking cue (context), and we did not have a measure of how context-based responding may have contributed to the observed responses to saccharin at test. In principle, one way to resolve this problem is to use separate contexts for conditioning and testing. However, our previous studies of latent inhibition using this procedure (López et al., 2010) suggest that the IO infusion itself and the chamber used to observe/record the orofacial reactions is the dominant contextual feature (see also Dwyer, Gasalla, & López, 2013).

TABLE 1

Therefore, Experiment 1 (see Table 1) evaluated the potential of the contextual cues alone (i.e., in the absence of the flavor) to display conditioned orofacial reactions, as well as the degree to which the context blocks conditioned orofacial reactions to the flavor cue. Two groups of rats (Groups Pre-Li and Pre-Sal) were given context-LiCl pairings whereas other two groups (Groups Non-Li and Non-Sal) received saline injections during pretraining. In the saccharin conditioning sessions using the same context, subjects in Groups Pre-Li and Non-Li were injected with LiCl after an intraoral infusion of saccharin whereas those in Groups Pre-Sal and Non-Sal were given saline injections. In the orofacial reactivity test, each subject was tested with the context alone, an intraoral infusion of saccharin, and finally, with an infusion of water, while their orofacial reactions were recorded. It was expected that rats pretrained with LiCl before conditioning of saccharin in compound with the context (Group Pre-Li) would both display aversive orofacial reactions when exposed to the context alone, and that they would display fewer aversive orofacial reactions when infused with the saccharin solution during testing compared to the controls.

Method

Subjects. Thirty two male Wistar rats, approximately 90 days old and with a mean free-feeding weight of 331 g (range, 220-393 g) at the start of the experiment, were used for the present study. Upon arrival, they were housed individually in standard plastic cages in a colony room maintained on a 12-h light/dark cycle (lights on at 08:00 h) and at an ambient temperature of 21º C. All experimental manipulations took place during the light phase. Throughout the experiment, rats were maintained on a water deprivation-schedule as described below. Food was always available in the home cages. All behavioral procedures were conducted in accordance with guidelines of the European Council Directive

(86/609/EEC) and Spanish regulation RD-1201/2005 regarding the care and use of laboratory animals.

Fluids and apparatus. The fluids used were solutions of lithium chloride (0.15 M LiCl), isotonic saline (0.9% NaCl solution), and saccharin (0.1% w/v). LiCl and NaCl were administered intraperitoneally (i.p.) at a volume of 10 ml/kg of body weight. The saccharin solution was infused directly into the mouth of the subject through an oral cannula implanted prior to the experiment, the details of which are described below.

The behavioral procedures took place in a conditioning chamber located in a dark room. The chamber was made of clear Plexiglas sides (26 cm x 23 cm x 14 cm) with a dark lid, and was placed on a table with a clear Plexiglas top. Two 50-Watt white lights on each side of the table provided a light illumination. A mirror beneath the chamber on a 45º angle facilitated viewing of the ventral surface of the rat during the intraoral infusion. Fluids were administered to the animals through an infusion pump (KD Scientific) connected to the implanted cannula. While the rats were infused with the fluids, their orofacial responses were recorded using a video camera (Sony Optical 20 X) connected to a computer. The videos were manually scored using the Observer XT 9.0 (Noldus Information Technology, Sterling, VA) event recording program.

Based on the procedure followed by Parker (1984; 1995), and as previously used in our lab (Gasalla, Begega, Soto, Dwyer, & López, 2016; Lopez et al., 2010), the aversive behaviors scored included the frequency of the responses of gaping (rapid, large-amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward) and paw treading (forward and backward movement of the forepaws in synchronous alternation). These scores were summed to provide a total aversive response score. The appetitive responses scored were tongue protrusions (extension of the tongue out the mouth), mouth

movements (movement of the lower mandible without opening the mouth), and paw licks (midline extension of the tongue directed to the forepaws). The number of seconds that the rats displayed the responses was used as the appetitive response score. Appetitive and aversive responses were scored on different scales (duration vs frequency) because they display very different properties: appetitive responses are typically displayed over extended periods of time, while aversive responses occur as isolated behaviors (Berridge, 2000). The videos were scored by two raters blind to the experimental groups.

Cannulation surgery. The rats were surgically implanted with an intraoral cannula using a very similar method to that described in Parker (1995). The surgical anesthesia preparation included administration of an i.p. injection of ketamine (50 mg/kg) combined with medetomidine hydrochloride (0.15 mg/kg), a drug with analgesic properties. Following surgery, the rats were administered ketoprofen (1.5 mg/kg, s.c.), an anti-inflammatory drug, and the antibiotic enrofloxacin (0.3 mg/kg, s.c.). In order to implant the cannula a thin-walled 15-gauge stainless steel needle was inserted at the back of the neck, directly subcutaneously around the ear and brought out behind the first molar inside mouth. A length of intramedic polyethylene tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was then run through the needle after which the needle was removed. Two square elastic discs were placed over the tubing and drawn to the exposed skin at the back of the neck for the purpose of stabilizing the cannula. The tubing was held secure in the oral cavity by an O-ring, which was sealed behind the tubing prior to cannulation surgery. Following surgery, rats were monitored for three days and had their cannula flushed daily with chlorhexidine to prevent infection. For the purpose of fluid infusion, the cannula was connected to the infusion pump by slipping the tubing of the cannula inside a second polyethylene tubing (inner diameter 1.19 mm; outer diameter 1.70 mm) attached to the infusion pump.

Procedure. Three rats lost their cannula during the experiment and were removed from the sample. The remaining animals were randomly assigned to four groups: Group Pre-Li (n=7); Group Non-Li (n=8); Group Pre-Sal (n=7); and Group Non-Sal (n=7). Three days after the surgery, the rats were placed on a water deprivation-schedule, comprising 1-h access to water each day, given approximately 2 h after the experimental sessions. Throughout the experiment, this water deprivation regime was maintained (unless otherwise noted).

On each of 4 pre-training sessions (see Table 1), rats were placed in the conditioning chamber, where they spent 5 min before being injected with lithium (0.15 M; 10 ml/kg) (Group Pre-Li and Pre-Sal) or physiological saline (0.9%; 10 ml/kg) (Group Non-Li and Non-Sal). Rats spent 60 min after the injection in the conditioning chamber before being returned to the home cage. After the second and fourth pre-training sessions, the animals received a water recovery day in their home cages. On the next day, the rats were habituated to the infusion procedure. They were placed in the conditioning chamber (the orofacial reactivity apparatus) with their cannula attached to the infusion pump for fluid delivery. After a period of 5 min, water was infused into their intraoral cannula for 1 min at the rate of 1 ml/min in order to habituate them to this fluid delivery method.

The next four days constituted the saccharin conditioning phase. The rats received two conditioning trials separated by a recovery day during which they were given water in their home cages. On each of the two conditioning trials, the animals were placed in the conditioning chamber and intra-orally infused with 0.1% saccharin for 5 min at a rate of 1 ml/min while their orofacial responses were recorded. Immediately following the fluid infusion, the rats in Groups Pre-Li and Non-Li were injected (i.p.) with LiCl whereas those in Groups Pre-Sal and Non-Sal received an injection of physiological saline. After the injections, the animals were kept 60 min in the conditioning chamber before being returned to the home cages.

The orofacial reactivity test occurred the next day. This test was divided into three 2.5 min-periods. During this session, each rat was placed in the conditioning chamber for 2.5 min while their orofacial responses were recorded; for half of the animals they were then intraorally infused with the saccharin solution (0.1%) for 2.5 min at a rate of 1 ml/min, and finally, with water (1 ml/min) for another 2.5 min; for the remaining animals, the order of saccharin and water infusion was reversed. During the fluid infusions the rats´ orofacial responses were recorded. On the next three days, consumption tests were administered. On each of these sessions, the rats were given access to a drinking tube containing the saccharin solution for 15 min in their home cages, and the amounts consumed were measured (by weight). Rats were given supplementary water for 60 min in their home cages at the end of each of these tests.

Data analysis. Aversive and appetitive orofacial reactions during conditioning were separately analyzed with 2 (pretraining: context pretrained vs not pretrained) × 2 (conditioning: saccharin paired with LiCl vs saline) × 2 (trial) mixed ANOVAs. The orofacial reactivity scores for the context alone, saccharin infusion, and water infusion during testing were analyzed by separate 2 (pretraining) × 2 (conditioning) ANOVAs. A 2 (pretraining) × 2 (conditioning) × 3 (trial) mixed ANOVA was used to examine the consumption data from the final bottle tests. For the ANOVA analyses we report partial eta squared as our measure of effect size with 95% confident intervals (CIs) using the procedure described by Steiger (2004). For contrast and t-test analyses, we report effect sizes as the Mean Difference with CIs calculated using Exploratory Software for Confident Intervals (ESCI, Cumming, 2013). All tests reported here used a criterion for significance of $p = 0.05$. The inter-rater reliability (r´s > 0.87) for each behavior scored was highly significant.

Results and discussion

Figure 1 (panel A) shows the mean number of aversive orofacial reactions elicited by the infusion of saccharin during the conditioning phase. On trial 2, rats in Group Non-Li displayed more aversive orofacial reactions to saccharin than the rats from the other three groups. A mixed ANOVA revealed significant main effects of trial $F(1,25) = 27.87$, $MSE = 4.25$, $p < .001$, $\eta_p^2 = .53$, 95% CI [.23, .69], pretraining $F(1,25) = 5.94$, $MSE = 5.38$, $p = .022$, $\eta_p^2 = .19$, 95% CI [0, .32], and conditioning $F(1,25) = 38.22$, $MSE = 5.38$, $p < .001$, $\eta_p^2 = .60$, 95% CI [.32, .74]. The interactions involving these factors were all significant: pretraining × trial $F(1,25) = 30.72$, $MSE = 4.25$, $p < .001$, $\eta_p^2 = .55$, 95% CI [.25, .70]; conditioning × trial $F(1,25) = 50.75$, $MSE = 4.25$, $p < .001$, $\eta_p^2 = .67$, 95% CI [.41, .78]; pretraining × conditioning $F(1,25) = 14.76$, $MSE = 5.38$, $p = .001$, $\eta_p^2 = .37$, 95% CI [.09, .57]; and pretraining × conditioning × trial $F(1,25) = 15.69$, $MSE = 4.25$, $p = .001$, $\eta_p^2 = .39$, 95% CI [.10, .59]. A simple effect analysis of the triple interaction revealed that Group Non-Li displayed significantly more aversive reactions in the second conditioning trial than the first $F(1,25) = 132.84$, $MSE = 2.27$, $p < .001$, $\eta_p^2 = .84$, 95% CI [.69, .90], but no other group showed a change in the number of aversive reactions between trials (largest $F(1,25) = 2.84$, $MSE = 7.34$, $p = .104$ for group Pre-Sal). In addition, a simple effect analysis of the pretraining × trial interaction revealed that the groups which had received context-LiCl pairings displayed more aversive responses on the first conditioning trial ($F(1,25) = 7.31$, $p = .012$, $\eta_p^2 = .23$, 95% CI [.01, .46]).

FIGURE 1

The mean duration (in seconds) of appetitive orofacial reactions elicited by the infusion of saccharin during the conditioning phase is presented in Table 2. While there was a significant reduction in appetitive reactions in Groups Pre-Li and Non-Li compared with Groups Pre-Sal and Non-Sal, the reduction in appetitive reactions was larger in Group Non-Li than Group Pre-Li. The ANOVA conducted with these scores revealed significant main effects of trial $F(1,25) = 154.99$, $MSE = 853.75$, $p < .001$, $\eta_p^2 = .86$, 95% CI [.73, .91], and conditioning $F(1,25) = 108.65$, $MSE = 2098.43$, $p < .001$, $\eta_p^2 = .81$, 95% CI [.64, .88], but not a significant effect of pretraining $F(1,25) = .33$, $MSE = 2098.43$, $p = .569$, $\eta_p^2 = .01$, 95% CI [0, .19]. The interactions involving these factors were all significant: pretraining × trial $F(1,25) = 10.29$, $MSE = 853.76$, $p = .004$, $\eta_p^2 = .29$, 95% CI [.04, .51]; conditioning × trial $F(1,25) = 174.23$, $MSE = 853.76$, $p < .001$, $\eta_p^2 = .87$, 95% CI [.75, .92] but conditioning × pretraining $F(1,25) = .40$, $MSE = 1049.21$, $p = .509$, $\eta_p^2 = .18$, 95% CI [0, .2]; and pretraining × conditioning × trial $F(1,25) = 9.25$, $MSE = 853.76$, $p = .005$, $\eta_p^2 = .27$, 95% CI [.03, .50]. A simple effect analysis of the triple interaction revealed that both conditioned groups, Group Pre-Li and Non-Li, showed significantly fewer appetitive reactions in the second conditioning trial than the first, $F(1,25) = 91.41$, $MSE = 1219.72$, $p < .001$, $\eta_p^2 = .78$, 95% CI [.59, .86] and $F(1,25) = 281.65$, $MSE = 1732.62$, $p < .001$, $\eta_p^2 = .92$, 95% CI [.84, .95], respectively, but the non-conditioned groups did not show a change in the duration of appetitive reactions between trials (largest $F(1,25) = .793$, $MSE = 1219.72$, $p = .391$, $\eta_p^2 = .03$, 95% CI [0, .23] for group Pre-Sal). Moreover, Group Non-Li displayed less appetitive reactions on trial 2 than did Group Pre-Li, $F(1,25) = 5.21$, $MSE = 1732.62$, $p = .031$, $\eta_p^2 = .17$, 95% CI [0, .41]. In addition, a simple effect analysis of the pretraining × trial interaction

revealed that the groups which had received context-LiCl pairings displayed fewer appetitive responses to saccharin on the first conditioning trial ($F(1,25) = 5.92$, $p = .022$, $\eta_p^2 = .19$, 95% CI [.01, .43]). The fact that appetitive reactions to saccharin were high, and aversive reactions low, on the first saccharin conditioning trial after prior context-LiCl pairings suggests that saccharin elicits unconditioned appetitive responses which obscured conditioned aversive responses to the context. However, groups which had received context-LiCl pairings displayed both fewer appetitive reactions and more aversive reactions than groups that had not, indicating an influence of prior context training at the outset of the conditioning phase.

TABLE 2

Panel B of Figure 1 shows the mean number of aversive reactions displayed by the various groups during the orofacial reactivity test. Analysis of the rates of aversive reactions elicited by the context alone revealed significant main effects of pretraining, $F(1,25) = 18.32$, $MSE = 23.54$, $p < .001$, $\eta_p^2 = .42$, 95% CI [.12, .61] and conditioning, $F(1,25) = 27.74$, $MSE = 23.54$, $p < .001$, $\eta_p^2 = .53$, 95% CI [.22, .69], as well as a significant interaction between these two factors, $F(1,25) = 4.94$, $MSE = 23.54$, $p = .036$, $\eta_p^2 = .16$, 95% CI [0, .40]. Simple effect analysis of the interaction revealed that Group Pre-Li showed more aversive reactions than Group Pre-Sal $F(1,25) = 27.19$, $MSE = 23.54$, $p < .001$, $\eta_p^2 = .52$, 95% CI [.22, .68], and Group Non-Li showed more aversive reactions than Group Non-Sal $F(1,25) = 4.80$, $MSE = 23.54$, $p = .038$, $\eta_p^2 = .16$, 95% CI [0, .40]. These reflect the fact that pairing the context with LiCl resulted in conditioned aversive reactions to that context regardless of whether LiCl was injected in the pretraining or conditioning phases (or both).

Analysis of the number of aversive reactions displayed by the rats during the intraoral infusion of saccharin in the orofacial reactivity test[3] revealed significant main effects of pretraining, $F(1,25) = 5.31$, $MSE = 20.21$, $p = .030$, $\eta^2_p = .17$, 95% CI [0, .41], and conditioning, $F(1,25) = 20.22$, $MSE = 20.21$, $p < .001$, $\eta^2_p = .45$, 95% CI [.15, .63], as well as a significant pretraining by conditioning interaction, $F(1,25) = 5.31$, $MSE = 20.21$, $p = .030$, $\eta^2_p = .17$, 95% CI [0, .41]. Simple effect analysis of the interaction revealed that Group Non-Li displayed more aversive reactions to the saccharin solution than Group Pre-Li, $F(1,25) = 10.98$, $MSE = 20.21$, $p = .003$, $\eta^2_p = .30$, 95% CI [.04, .52], but Group Non-Sal and Pre-Sal did not differ, $F < 1$. This confirms the blocking of aversive reactions to saccharin by context pretraining. Finally, analysis of the number of aversive reactions during the water infusion revealed a main effect of conditioning, $F(1,25) = 14.63$, $MSE = 15.24$, $p = .001$, $\eta^2_p = .37$, 95% CI [.08, .57], but no effect of pretraining nor any interactions between these two factors ($Fs < 1$).

Table 2 presents the mean duration of appetitive reactions displayed by the rats during the infusion of saccharin in the orofacial reactivity test. The ANOVA performed with these scores revealed significant main effects conditioning, $F(1,25) = 850.09$, $MSE = 150.57$, $p < .001$, $\eta^2_p = .97$, 95% CI [.94, .98], but no effect of pretraining, $F(1,25) = 0.59$, $MSE = 150.57$, $p = .449$, $\eta^2_p = .02$, 95% CI [0, .22], or significant pretraining by conditioning interaction, $F(1,25) = 1.29$, $MSE = 150.57$, $p = .267$, $\eta^2_p = .04$, 95% CI [0, .26].

[3] In order to analyse if the aversive reactions are subject to 'fatigue' over the test session, data from saccharin and water infusions at test was analysed including an order factor, that is, if the saccharin was administered before or after the water infusion. There were no effects of order on aversive responses to water infusions, $F(1,27) = .11$, MSE = 22.29, $p = .747$, $\eta^2_p = .01$, 95% CI [0, .14], or to saccharin infusion, $F(1,27) = .04$, MSE = 43.09, $p = .850$, $\eta^2_p = .01$, 95% CI [0, .11].

Panel C of Figure 1 shows the mean amount of saccharin solution consumed during the subsequent consumption tests. Fluid intake by groups Pre-Li and Non-Li increased over the trials, whereas consumption intake by groups Pre-Sal and Non-Sal remained high across the sessions. There were significant main effects of trial $F(2,50) = 17.39$, $MSE = 5.25$, $p < .001$, $\eta_p^2 = .41$, 95% CI [.19, .55], pretraining $F(1,25) = 4.82$, $p = .038$, $\eta_p^2 = .16$, 95% CI [0, .40], conditioning $F(1,25) = 74.49$, $MSE = 18.48$, $p < .001$, $\eta_p^2 = .75$, 95% CI [.53, .83], and a significant pretraining × trial interaction, $F(2,50) = 3.27$, $MSE = 5.25$, $p = .046$, $\eta_p^2 = .12$, 95% CI [0, .35]. There was neither a significant conditioning × trial interaction, nor a significant interaction of the three factors ($Fs < 1$). The pretraining × conditioning interaction also was not significant $F(1,25) = 1.46$, $MSE = 18.48$, $p = .238$. The main effect of pre-training, and the pretraining by trial interaction, are consistent with a blocking of saccharin aversion by the context, this was confirmed by a 2-way ANOVA examining only the groups which received saccharin paired with LiCl in the conditioning phase: this revealed significant main effects of pretraining, $F(1,13) = 7.43$, $MSE = 69.63$, $p = .017$, $\eta_p^2 = .36$, 95% CI [.01, .58], and trial $F(2,26) = 19.55$, $MSE = 5.13$, $p < .001$, $\eta_p^2 = .60$, 95% CI [.30, .73], as well as a significant trial by group interaction $F(2,26) = 4.31$, $MSE = 5.13$, $p = .024$, $\eta_p^2 = .25$, 95% CI [0, .42].

In summary, when rats received saccharin-LiCl training without pretraining to the conditioning context (Group Non-Li) they displayed few aversive reactions to the context alone, and high levels of aversive reactions to saccharin. In contrast, when rats received context-LiCl pairings prior to saccharin-LiCl training (Group Pre-Li) they displayed a high rate of aversive reactions to the context alone, but few aversive reactions to saccharin. While appetitive orofacial reactions were at floor levels during test for both groups receiving

saccharin-LiCl pairings, the rate of reduction in appetitive responses to saccharin across conditioning was lower in Group Pre-Li than Non-Li. The comparison of responding to the saccharin infusion and to the context alone between these two groups demonstrates that pairing a context with LiCl can result in the context eliciting aversive reactions, the blocking of aversive orofacial reactions to a taste conditioned in that context, and (notwithstanding floor effects on test) blocking of reductions in appetitive orofacial reactions to the conditioned taste. That is not to say that saccharin-LiCl pairings were entirely without effect when following context-LiCl pairings: Group Pre-Li displayed fewer appetitive reactions and more aversive reactions at test than did groups which did not receive saccharin-LiCl pairings. So blocking of taste-LiCl learning by the context was partial rather than complete. We would note that this is not a standard blocking test, because the blocked cue (saccharin) is tested in combination with the blocking cue (context). However, this does not affect the conclusions reached here because this design would be likely to underestimate any true blocking effect as responding to the blocking cue might mask any reduction in responding to the blocked cue. In light of the overall aim of these studies, these results confirm that context stimuli can elicit conditioned aversive orofacial reactions in the absence of any flavor component, and demonstrate for the first time that context cues can also interfere with the conditioned reduction in palatability of a taste receiving aversion training in that context.

Experiment 2

Because of the theoretical importance of the novel results from Experiment 1, we sought to replicate and extend them by examining whether the context-blocking effect on conditioned changes in taste palatability depends on the strength of the context-LiCl association. That is, we examined context blocking as a function of whether or not the context-LiCl association has been extinguished by exposing the rats to the context alone

before saccharin was conditioned in compound with the context. It is known that extinction reduces the strength of the context-LiCl association and therefore the ability of the context to block a subsequent taste aversion as assessed by a consumption test (Batson & Best, 1979; Boakes, Westbrook, Elliott, & Swinbourne, 1997; Iguchi, Fukumoto, Sawa, & Ishii, 2014). Based on this finding, it would be expected that extinction would also weaken the association between the context and nausea, allowing for the establishment of conditioned reductions in palatability for the saccharin solution when it is paired with LiCl in the extinguished context.

The design of this experiment is summarized in Table 1. Two groups of rats, Pre and Pre-Ext, were administered with LiCl in a distinctive context (the conditioning apparatus) four times. Rats in Group Pre-Ext were then exposed to the context four times and given saline injections in order to extinguish its ability to elicit conditioned nausea, whereas rats in Group Pre received the saline in the home cages (a third group of rats, Group Non, was given saline injections during pretraining and extinction sessions in the context). Following this, all rats received two conditioning trials in which an infusion of saccharin in the presence of the contextual cues was paired with LiCl. The affective responses to the context alone and to saccharin was then examined in the orofacial reactivity test.

Method

Subjects, fluids, and apparatus. Thirty male Wistar rats, approximately 90 days old, weighing from 273 to 379 g at the start of the experiment served as subjects. Except otherwise stated, deprivation conditions, apparatus, and other procedural details were the same as in Experiment 1. Each subject was implanted with an oral cannula using the procedure described in Experiment 1. The flavor used during the experiment was a 0.1 % (w/v) saccharin solution. The rats were injected with either 10 ml/kg of 0.15 M LiCl or physiological saline (10 ml/kg). Two rats lost their cannula during the experiment, so that the

number of subjects in each group was: Group Pre (n=8); Group Non (n=10); and Group Pre-Ext (n=10).

Procedure. The pretraining phase was similar to that of Experiment 1 (see Table 1). In each of four trials (one per day), the rats were placed in the conditioning chamber for 2.5 min while their orofacial responses were video-recorded. Immediately afterwards the subjects were injected with LiCl (Group Pre and Group Pre-Ext) or saline (Group Non). The rats were returned to the conditioning chamber for 60 min after the injection. They received a water recovery day after the second and fourth pretraining trials. The next four sessions constituted the extinction phase. During these sessions, the rats in Group Non and Group Pre-Ext were placed in the conditioning chamber for 2.5 min and their orofacial responses recorded before being injected with saline (10 ml/kg; 0.9 % NaCl solution). They were returned to the conditioning apparatus for 60 min after the injection. The animals in Group Pre received a saline injection in each session before being returned to their home cages. After completion of this phase, the rats were habituated to the orofacial reactivity procedure by infusion with water for a period of 1 min at the rate of 1 ml/min.

On each of the following two sessions, the rats received the saccharin conditioning trials. On these sessions, the rats were placed in the conditioning chamber and intraorally infused with 0.1 % saccharin for 5 min while their reactions were video-recorded. Immediately following the saccharin infusion, the rats were all injected with LiCl. The rats were kept for 60 min in the conditioning chamber before being returned to their home cages. After a recovery day with water in the home cages, the orofacial reactivity test was administered. As in Experiment 1, each rat was placed in the conditioning chamber for 2.5 min while their orofacial responses were recorded; they were then intraorally infused with saccharin (0.1%) for 2.5 min at a rate of 1 ml/min, and finally, with water (1 ml/min) for

another 2.5 min. The sequence of infusions (saccharin and water) was counterbalanced as in Experiment 1. No consumption test was conducted in this experiment.

Data analysis. The behaviors scored during pretraining were analyzed with a 3 (group) × 4 (trial) mixed ANOVA. During the extinction phase a similar ANOVA was used (but with only 2 levels of the group factor because Group Pre did not received any experimental manipulations in this phase). The data during the conditioning trials was analyzed by means of a 3 (group) × 2 (trial) mixed ANOVA. The orofacial reactivity scores for context alone, saccharin infusion, and water infusion during testing were analyzed by separate one-way ANOVAs with group as between-group factor. The inter-rater reliability (r´s > 0.93) for each behavior scored was highly significant.

Results and discussion

Panel A of Figure 2 (left-hand side) shows the mean number of aversive orofacial reactions displayed by the rats to contextual cues on each of the four pretraining sessions. The number of aversive reactions increased over the trials to the same extent in Groups Pre and Pre-Ext. The 3 × 4 ANOVA conducted on these data revealed significant main effects of trial and group ($F(3,75) = 37.69$, $MSE = 4.20$, $p < .001$, $\eta_p^2 = .60$, 95% CI [.44, .69], and $F(2,25) = 16.77$, $MSE = 5.64$, $p < .001$, $\eta_p^2 = .57$, 95% CI [.26, .71] respectively) and a significant interaction between trial and group, $F(6,75) = 10.01$, $MSE = 4.20$, $p < .001$, $\eta_p^2 = .44$, 95% CI [.23, .54]. Follow-up contrast analysis of the interaction revealed no differences between groups on trials 1 and 2 (largest $t(25) = 1.23$, $p = .229$, Mean Difference = 0.68, 95% CI [-.45, 1.80] for the difference between Group Non against Groups Pre and Pre-Ext combined), while Group Non displayed fewer aversive reactions on trials 3 and 4 than either of Groups Pre and Pre-Ext combined ($t(25) = 2.76$, $p = .01$, Mean Difference = 5.37, 95% CI [1.37,

9.38] and $t(25) = 5.75$, $p < .001$, Mean Difference = 15.55, 95% CI [9.99, 21.11] respectively), which did not themselves differ ($t(25) = 0.32$, $p = .751$, Mean Difference = 0.37, 95% CI [-2.03, 2.78] and $t(25) = 1.20$, $p = .241$, Mean Difference = 1.95, 95% CI [-1.39, 5.29] respectively for trials 3 and 4).

FIGURE 2

Figure 2A (right-hand side) presents the mean number of aversive orofacial reactions expressed by rats in Groups Pre-Ext and Non during the extinction phase. The 2 (group) × 4 (trial) ANOVA of the number of aversive reactions during these sessions revealed significant main effects of group, $F(1,18) = 24.77$, $MSE = 3.56$, $p < .001$, $\eta_p^2 = .58$, 95% CI [.22, .74] and trial, $F(3,54) = 11.56$, $MSE = 2.98$, $p < .001$, $\eta_p^2 = .39$, 95% CI [.16, .52], and a significant group × trial interaction, $F(3,54) = 11.56$, $p < .001$, $\eta_p^2 = .39$, 95% CI [.16, .52]. Analysis of group differences for each trial revealed that during extinction trials 1 and 2, Group Pre-Ext displayed significantly more aversive reactions than Group Non ($t(18) = 4.56$, $p < .001$, Mean Difference = 5.80, 95% CI [3.13, 8.47] and $t(18) = 2.47$, $p = .024$, Mean Difference = 2.10, 95% CI [0.36, 3.88] respectively), but that the groups did not differ on extinction trials 3 and 4 ($t(18) = 1.25$, $p = .229$, Mean Difference = 0.50, 95% CI [-.34, 1.34] for trial 3 and a t-test could not be computed as all values were 0 for trial 4).

During the saccharin conditioning phase Groups Non and Pre-Ext displayed significantly more aversive reactions than Group Pre as conditioning proceeded. Figure 2 (panel B) presents the mean number of conditioned aversive reactions elicited by the infusion of saccharin during the two conditioning trials. The 3 × 2 ANOVA revealed significant main effects of group, $F(2,25) = 3.65$, $MSE = 6.87$, $p = .041$, $\eta_p^2 = .22$, 95% CI [0, .43] and trial,

$F(1,25) = 73.94$, $MSE = 6.87$, $p < .001$, $\eta_p^2 = .75$, 95% CI [.53, .83], and a significant interaction between these factors, $F(2,25) = 3.55$, $MSE = 6.87$, $p = .044$, $\eta_p^2 = .22$, 95% CI [0, .43]. Follow-up contrast analysis of the interaction revealed no differences between groups on trial 1 (largest $t(25) = 1.18$, $p = .250$, Mean Difference = 0.10, 95% CI [-.07, 0.27] for the difference between Groups Pre-Ext and Non), and that Group Pre displayed fewer aversive reactions on trial 2 than Groups Non and Pre-Ext combined ($t(25) = 2.63$, $p = .014$, Mean Difference = 8.15, 95% CI [1.78, 14.52]) , which did not themselves differ ($t(25) = 0.54$, $p = .592$, Mean Difference = 0.90, 95% CI [-2.52, 4.33]). The duration of appetitive reactions displayed by the animals during the conditioning phase is presented in Table 2. The 3 × 2 ANOVA revealed significant main effect of trial $F(1,25) = 876.78$, $MSE = 619.44$, $p < .001$, $\eta_p^2 = .97$, 95% CI [.94, .98] but no significant effect of group, $F(2,25) = 1.82$, $MSE = 701.37$, $p = .182$, $\eta_p^2 = .12$, 95% CI [0, .29] or a significant interaction between these factors, $F(2,25) = 2.83$, $MSE = 619.44$, $p = .078$, $\eta_p^2 = .18$, 95% CI [0, .33]. Although the interaction did not reach standard levels of significance, Group Pre displayed both less appetitive reactions on conditioning trial 1 (t(25) = 2.14, p = .043, Mean Difference = 35.40, 95% CI [1.26, 69.54]) and a smaller reduction between conditioning trials 1 and 2 (t(25) = 2.25, p = .034, Mean Difference = 37.56, 95% CI [3.18, 71.94]) than did group Pre-Ext (although Group Non did not differ from either of the other two groups on conditioning trail 1 or two, largest t(25) = 1.54, p = .137, Mean Difference = 23.98, 95% CI [-56.17, 8.20], for the difference between Groups Pre-Ext and Non on trial 1).

Panel C of Figure 2 presents the mean number of aversive reactions for the different groups during the orofacial reactivity tests. The one-way ANOVA conducted with the aversive reactions elicited by the context alone revealed a significant effect of group, $F(2,25)$

= 4.54, *MSE* = 29.22, $p = .021, \eta_p^2 = .26$, 95% CI [0, .47]. Follow-up contrast analysis revealed that Group Pre displayed more aversive reactions to the context than Groups Non and Pre-Ext combined ($t(25) = 2.99$, $p = .006$, Mean Difference = 4.52, 95% CI [1.41, 7.63]), which did not themselves differ ($t(25) = 0.41$, $p = .683$, Mean Difference = 2.42, 95% CI [-9.96, 14.80]), indicating that the LiCl-associated context acquired the ability to elicit conditioned aversive responses, and that non-reinforced exposures to the context after lithium-context pairings resulted in attenuated reductions in affective value. Analysis of the number of aversive reactions during the intraoral infusion of saccharin revealed a significant effect of group, $F(2,25) = 4,61$, *MSE* = 16.24, $p = .019, \eta_p^2 = .27$, 95% CI [.01, .47]. Follow-up contrast analysis revealed that Group Pre displayed fewer aversive reactions to saccharin than Groups Non and Pre-Ext combined ($t(25) = 3.06$, $p = .005$, Mean Difference = 10.30, 95% CI [3.38, 17.22]), which did not themselves differ ($t(25) = 0.11$, $p = .913$, Mean Difference = 0.20, 95% CI [-3.62, 4.02]). These results suggest that the reduction in palatability of LiCl-paired saccharin was attenuated when taste-LiCl training occurred in a context able to elicit aversive orofacial reactions. The ANOVA also revealed that there was no significant effect of group on the number of aversive reactions displayed by the rats during the water infusion ($F < 1$). Finally, appetitive reactions elicited by the saccharin during the orofacial reactivity test were at floor levels (see table 2) and ANOVA revealed no significant effect of group, $F(2,25) = 1.25$, *MSE* = 4.9, $p = .304, \eta_p^2 = .09$, 95% CI [.0, .29].

In summary, as in Experiment 1, animals that received context-LiCl pairings displayed aversive orofacial reactions to the context alone, and after saccharin-LiCl pairings in the same context displayed fewer aversive orofacial reactions to saccharin than animals which had not received context-LiCl pairings. Moreover, extinction of the context-LiCl association through context alone presentations removed this blocking effect. As in

Experiment 1, despite this blocking effect there was still some evidence of saccharin-LiCl learning as all groups showed an increase in aversive reactions and a decrease in appetitive reactions to saccharin across training. These results confirm that the observations from Experiment 1 are reliable, and demonstrate that the blocking effect depends on the current strength of the context-LiCl association and cannot be explained by non-specific effects of LiCl or context exposure alone.

General discussion

This study explored the role of contextual stimuli in LiCl-based aversion learning by examining (1) whether non-flavor contextual cues can elicit conditioned changes in affective responses, and (2) whether context-nausea associations could interfere with the establishment of conditioned changes in palatability to a flavor trained in a nausea-paired context. In both of Experiments 1 and 2, contextual cues alone (with no flavor component) that had been paired with LiCl elicited conditioned aversive orofacial responses. Moreover, in both experiments context-LiCl pairings subsequently reduced the number of aversive orofacial responses elicited by saccharin when it was trained with LiCl in the same context. The blocking effect was not total as there was evidence for increases in aversive reactions and decreases in appetitive reactions to saccharin even in the blocked groups. Experiment 2 demonstrated that this blocking effect was removed when context-LiCl associations were extinguished prior to saccharin-LiCl pairings, indicating that the blocking effect depended on the strength of the context-LiCl association. Although appetitive orofacial responses were at floor levels on test for all groups receiving saccharin-LiCl pairings, there was evidence (especially in Experiment 1) for blocking in that the rate of reduction of appetitive responses across training with saccharin was lower when this occurred in a LiCl-paired context. Finally, Experiment 1 confirmed that the blocking effect extended to consumption measures.

Taking first the demonstration of aversive orofacial reactions to a LiCl-paired context: Prior studies had provided inconsistent results, with one (Limebeer et al., 2008) reporting conditioned aversive reactions to contexts which had no flavor component, while another (Meachum & Bernstein, 1992) reporting aversive orofacial reactions only to contexts which had an explicit odor component (even though the context without an odor did elicit other conditioned reactions). The fact that we consistently observed aversive orofacial reactions to contexts (which were entirely without an explicit odor or flavor component) across both of Experiments 1 and 2 would appear to settle this issue. While it is not entirely clear why Meachum and Bernstein (1992) failed to observe aversive orofacial reactions to a context without an explicitly added odor, one possible contribution to the different patterns of results is the sensitivity of the behavioral scoring methods: Meachum and Bernstein relied on live observation of 6 animals at a time (scoring 10s of behavior for each rat every minute across a 30min test), while we (and Limebeer et al., 2008) recorded close-up video of individual animals for off-line scoring (including the facility for slow-motion observation).

Turning to the issue of context-blocking: As before mentioned, a number of previous studies (including Batson & Best, 1979; Krane, 1980; Kwok & Boakes, 2012; Rodríguez et al., 2000; Symonds & Hall, 1997; Symonds et al., 1998) have used measurements of consumption to demonstrate that LiCl-paired contexts can interfere with learning about taste stimuli paired with LiCl in those contexts. However, because none of these context-blocking studies assessed orofacial reactivity, they left open the possibility that the conditioned reduction in palatability typically observed in taste aversion might be impervious to interference from prior context-nausea learning. Again, the current results appear to settle this issue: In Experiment 1 saccharin-LiCl pairings given in a LiCl-paired context resulted in fewer aversive reactions and a slower reduction in appetitive reactions to saccharin, as well as greater subsequent consumption of saccharin, compared to controls trained in a context that

had not been paired with LiCl; Experiment 2 confirmed the attenuation of aversive orofacial reactions and extended it to show that extinction of the context prior to saccharin conditioning removed the effect, demonstrating that blocking depends on the strength of the context-LiCl association. Taken together, these results indicate that all aspects of taste aversion learning (in particular conditioned changes in taste palatability) are susceptible to interference from non-taste contextual stimuli[4]. We have already considered a number of demonstrations of context-blocking of consumption in flavor aversion that suggest learning about flavor and non-flavor cues interact. In addition, the ability of environmental cues can modulate conditioned changes in taste palatability has also been examined in other ways. For example, Brown, Penney, Skinner, and Martin (2011) used a context discrimination task where rats received a saccharin solution paired with LiCl injections in one context, alternating with presentations of the saccharin followed by saline in another context. They found that both consumption of saccharin, and orofacial responses to it, differed between the two contexts. More recently, Sticht, Leach, Wilson, and Parker (2015), demonstrated through a second-order conditioning procedure that flavors presented in a previously LiCl-paired context can themselves develop the ability to elicit aversive orofacial reactions.

[4] This conclusion holds regardless of whether a one- or two-dimensional interpretation of orofacial reactivity responses is preferred. On a one-dimensional characterization the conditioned change in palatability was lower in the blocking groups because saccharin moved from electing many appetitive to few appetitive reactions, but did not elicit many aversive responses (in contrast to the control groups where saccharin moved from electing many appetitive reactions to eliciting a large number of aversive responses). On a two dimensional characterization there was clear evidence of blocking of conditioned disgust from the aversive orofacial responses, and some evidence of blocking of the reduction in positive hedonic value as the rate of decrease in appetitive responses was slowed across conditioning (although this was somewhat obscured by floor effects). However the affective changes produced by taste aversion are characterized, prior context-LiCl pairings interfere with them through blocking.

While perhaps not the predominant perspective for specialists, the idea that flavor aversion learning is based on highly specialized mechanisms for avoiding poisonous foods has both a long history and remains prevalent in the more general literature (e.g., Bures, Bermúdez-Rattoni, & Yamamoto, 1998; Buss, 2012; Domjan, 2005; Rozin & Kalat, 1971). This view initially derived from the demonstration that when a compound of audio-visual stimuli with a flavor predicted illness in rats, only the flavor was subsequently avoided, while the same compound stimulus predicting footshock led to the audio-visual stimuli being avoided (Garcia et al., 1955; Garcia & Koelling, 1966). Moreover, palatable flavors rejected after being pairing with illness elicit aversive orofacial reactions, while palatable flavors rejected because they predict non-illness aversive consequences such as shock do not elicit the same aversive reactions (Pelchat, et al., 1983). If flavor-aversion is based on a unique process, then non-flavor cues paired with nausea should not elicit the full range of conditioned responses (in particular conditioned changes in affective responses) – but context cues do elicit these reactions. If flavor-aversion is based on a selective process, then it should be unaffected by interference from non-flavor cues – but context cues do block nausea-induced reductions in taste palatability. Thus the current results confirm that a strong version of the idea that taste aversion learning is a special process is untenable.

That said, it is clear that there are at least some differences between flavored cues and those from other modalities with respect to learning about nausea-producing outcomes. Garcia's seminal studies (e.g. Garcia et al., 1955; Garcia & Koelling, 1966) elegantly demonstrated that it was easier to establish flavor-nausea learning than flavor-shock learning (and the opposite was true for audio-visual stimuli). While this was taken to be a qualitative difference between flavor and other cue modalities, the fact that we have clear evidence for context-nausea learning questions such an interpretation. Our results are certainly consistent with differences in the rate of acquisition between flavor and context cues – for example, a

comparison of the rates of acquisition of aversive orofacial reactions to the context in Figure 2A and to saccharin in Figures 1A and 2B suggest that a single saccharin-LiCl pairing was sufficient to elicit some evidence of aversive reactions while at least two pairings were needed to establish aversive responding to the context (in addition, responding to the context on test, after 6 context-LiCl pairings, was roughly equivalent to that of saccharin after 2 saccharin-LiCl pairings – see Figures 1B and 2C). Similarly, the demonstration of context-based second order conditioning by Sticht et al. (2015) was only apparent after 4 or 8 context-LiCl pairings and not after 2 context-LiCl pairings. Therefore, the apparent qualitative distinction in Garcia's classic work may simply be the product of insufficient training. There might also be differences between modalities in response to timing manipulations: For example flavor-shock learning can be enhanced by using a delayed presentation of the shock, but delay has the opposite effects on audio-visual cues (Krane & Wagner, 1975). Thus there may be a tendency to conflate differences of degree with differences of kind.

Finally, there is evidence that nausea as an outcome results in somewhat different response patterns than do some other types of aversive stimulation. Nausea paired cues elicit aversive orofacial reactions, while cues paired with some other aversive outcomes appear to be rejected without an increase in aversive reactions (Dwyer, Boakes, & Hayward, 2008; Parker, 2003, 2014; Parker, Limebeer, & Rana, 2009; but see also Lin et al. 2014; 2016 for analysis of the fact that many different aversive outcomes produce a reduction in appetitive orofacial responses and lick cluster size). Because these studies are typically conducted with flavor stimuli, it is possible that the response properties elicited by nausea-producing agents are attributed to flavor-nausea learning rather than to nausea itself.

Regardless of the reasons why the idea persists in the general literature that flavor aversion learning is based on highly selective cognitive processes, there is a breadth of

evidence inconsistent with this view. In demonstrating that conditioned aversive orofacial responses can be elicited by context cues and are not unique to flavors, and that context cues paired with nausea will attenuate through blocking the reduction in palatability of a nausea-paired taste, the current results exemplify and extend this evidence. Thus, our results are consistent with the operation of general associative mechanisms and the special properties of nausea itself rather than a selective mechanism for avoiding food poisoning: contextual, non-flavor, cues paired with LiCl appear to acquire the same conditioned aversive properties as do LiCl-paired flavors.

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Conflict of interest

The authors declare that they have no conflict of interest, financial or otherwise, related to this work.

References

Arthurs, J., Lin, J.-Y., Amodeo, L. R., & Reilly, S. (2012). Reduced palatability in drug-induced taste aversion: II. Aversive and rewarding unconditioned stimuli. *Behavioral Neuroscience, 126*(3), 433-444. doi:10.1037/a0027676

Baird, J. P., St. John, S. J., & Nguyen, E. A. N. (2005). Temporal and qualitative dynamics of conditioned taste aversion processing: Combined generalization testing and licking microstructure analysis. *Behavioral Neuroscience, 119*(4), 983-1003. doi:10.1037/0735-7044.119.4.983

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

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience and Biobehavioral Reviews, 24*(2), 173-198.

Berridge, K. C., & Grill, H. J. (1983). Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. *Behavioral Neuroscience, 97*(4), 563-573. doi:10.1037/0735-7044.97.4.563

Berridge, K. C., & Grill, H. J. (1984). Isohedonic tastes support a two-dimensional hypothesis of palatability. *Appetite, 5*(3), 221-231.

Best, M.R., Brown, E.R., & Sowell, M.K. (1984). Taste-mediated potentiation of noningestional stimuli in rats. *Learning and Motivation*, *15*, 244-258. doi: 10.1016/0023-9690(84)90021-3

Blaisdell, A. P., Gunther, L. M., & Miller, R. R. (1999). Recovery from blocking achieved by extinguishing the blocking CS. *Animal Learning & Behavior, 27*, 63-76.

Boakes, R.A., Westbrook, R.F., & Barnes, B.W. (1992). Potentiation by a taste of toxicosis-based context conditioning: Effects of varying the test fluid. *Quarterly Journal of Experimental Psychology, 45*B, 303-325. doi: 10.1037/0097-7403.23.1.56

Boakes, R.A., Westbrook, R.F., Elliott, M., & Swinbourne, A.L. (1997). Context dependency of conditioned aversions to water and sweet tastes. *Journal of Experimental Psychology: Animal Behavior Processes, 23*(1), 56-67. doi: 10.1037/0097-7403.23.1.56

Breslin, P. A. S., Grill, H. J., & Spector, A. C. (1992). A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium chloride pairings: a unidimensional account of palatability. *Behavioral Neuroscience, 106*(5), 820-836.

Brown, A.R., Penney, A.M., Skinner, D.M., & Martin, G.M. (2011). Aversive, appetitive and flavour avoidance responses in the presence of contextual cues. *Learning & Behavior, 39,* 95-103. doi: 10.3758/s13420-010-0008-x

Bures, J., Bermudez-Rattoni, F., & Yamamoto, T. (1998). Conditioned taste aversion. Memory of a special kind. Oxford: Oxford University Press.

Buss, D. M. (2012). Evolutionary psychology: The new science of the mind (4th ed.). Boston: Allyn & Bacon.

Cumming, G. (2013). Exploratory Software for Confidence Intervals - ESCI. Retrieved from www.thenewstatistics.com.

Davis, J. D. (1989). The microstructure of ingestive behavior. *Annals of the New York Academy of Sciences, 575*(1), 106-121.

Domjan, M. (1980). Ingestional aversion learning: Unique and general processes. *Advances in the study of behavior, 11*, 275-336.

Domjan, M. (2005). Pavlovian conditioning: A functional perspective. *Annual Review of Psychology, 56,* 179-206. doi: 10.1146/annurev.psych.55.090902.141409

Dwyer, D.M. (2012). Licking and liking: The assessment of hedonic responses in rodents. *Quarterly Journal of Experimental Psychology*, *65*, 371-394. doi: 10.1080/17470218.2011.652969

Dwyer, D. M., Boakes, R. A., & Hayward, A. J. (2008). Reduced palatability in lithium-and activity-based, but not in amphetamine-based, taste aversion learning. *Behavioral Neuroscience*, *122*(5), 1051. doi: 10.1037/a0012703

Dwyer, D.M., Gasalla, P. & López M. (2013). Nonreinforced flavor exposure attenuates the effects of conditioned taste aversion on both flavor consumption and cue palatability. *Learning & Behavior*, *41*, 390-401. doi: 10.3758/s13420-013-0114-x

Garcia, J., Hankins, W. G., & Rusiniak, K. W. (1974). Behavioral regulation of the milieu interne in man and rat. *Science, 185*(4154), 824-831.

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

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

Garcia, J., & Koelling, R. A. (1967). A comparison of aversions induced by X rays, toxins, and drugs in the rat. *Radiation Research Supplement, 7*, 439-450.

Gasalla, P., Begega, A., Soto, A., Dwyer, D. M., & López, M. (2016). Functional brain networks underlying latent inhibition of conditioned disgust in rats. *Behavioural Brain Research , 315*, 36-44. doi:http://dx.doi.org/10.1016/j.bbr.2016.07.051

Grill, H.C. & Norgren, R. (1978). The taste reactivity test: I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research*, *143*, 263-279. doi: 10.1016/0006-8993(73)90568-1

Iguchi, Y., Fukumoto, K., Sawa, K., & Ishii, K. (2014). Effects of extended context discrimination training and context extinction on transfer of context dependency of