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Lactose Malabsorption and Taste Aversion Learning

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Abstract

Consumption of foods can be suppressed by two feeding system defense mechanisms: conditioned taste aversion (CTA) or taste avoidance learning (TAL). There is a debate in the literature about which form of intake suppression is caused by various aversive stimuli. For instance, illness-inducing stimuli like lithium chloride are the gold standard for producing CTA and external (or peripheral) painful stimuli, such as footshock, are the traditional model of TAL. The distinction between CTA and TAL, which have identical effects on intake, is based on differential effects on palatability. That is, CTA involves a decrease in both intake and palatability, whereas TAL suppresses intake without influencing palatability. We evaluated whether lactose, which causes gastrointestinal pain in adult rats, produces CTA or TAL. Using lick pattern analysis to simultaneously measure intake and palatability (i.e., lick cluster size and initial lick rate), we found that pairing saccharin with intragastric infusions of lactose suppressed both the intake and palatability of saccharin. These results support the conclusion that gastrointestinal pain produced by lactose malabsorption produces a CTA, not TAL as had previously been suggested. Furthermore, these findings encourage the view that the CTA mechanism is broadly tuned to defend against the ingestion of foods with aversive post-ingestive effects.

Keywords

Lick pattern analysis; Palatability; Lactose; Conditioned taste aversion; Taste avoidance learning

1. Introduction

The present article is concerned with the nature of the learning that occurs when ingestion of a taste stimulus (conditioned stimulus; CS) is followed by the aversive internal effects (unconditioned stimulus; US) caused by lactose malabsorption. Taste learning with an aversive US can be categorized as either a conditioned taste aversion (CTA; for reviews see Barker, Best & Domjan, 1977; Braveman & Bronstein, 1985; Milgram, Krames & Alloway, 1977; Reilly & Schachtman, 2009) or as taste avoidance learning (TAL; Brett, 1977; Garcia & Koelling, 1966; Garcia, Kovner & Green, 1970; Parker, 1995; 2003; Pelchat, Grill, Rozin

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& Jacobs, 1983). Both types of learning cause a reduction in the amount consumed of the taste CS. However, CTA also involves a conditioned downshift in the palatability of the CS; no change in palatability occurs in TAL.

One method of assessing taste palatability in non-human animals involves detailed analysis of the patterns of licks that occur during voluntary consumption (e.g., Davis, 1989; Davis & Smith, 1992; Dwyer, 2012). A number of dependent measures can be extracted from the stream of licks, including two that are considered to accurately reflect palatability: lick cluster size (Davis, 1996; Davis & Perez, 1993; Davis & Smith, 1992; Higgs & Cooper, 1996; Katsuura, Heckman, & Taha, 2011; Spector, Klumpp, & Kaplan, 1998; Spector & Smith, 1984; Spector & St. John, 1998; for a review see Dwyer, 2012), and initial lick rate (Davis, 1998; Davis & Perez, 1993; Overduin, Figewicz, Bennett-Jay, Kittleson, & Cummings, 2012; Spector, Klumpp, & Kaplan, 1998). Lick pattern analysis has confirmed that lithium chloride, the quintessential laboratory US used to induce CTAs, causes a reduction in both intake and palatability (e.g., Arthurs, Lin, Amodeo & Reilly, 2012; Baird, St John & Nguyen, 2005; Dwyer, Boakes, & Hayward, 2008; Kent, Cross-Mellor, Kavaliers & Ossenkopp, 2002).

Using this method, we found that gallamine and hypertonic saline, each US known to cause a reduction of CS intake (Ionescu & Burešová, 1977; Lett, 1985; Sakai & Yamamoto, 1997), also conditionally lowers the palatability of the associate taste CS (Lin, Arthurs & Reilly, 2013). Gallamine is a neuromuscular blocking agent that causes transient pain and paralysis in muscle tissues (Cull-Candy & Miledi, 1983) and hypertonic saline is a laboratory model of visceral pain (Drewes, Babenko, Birket-Smith, Funch-Jensen & Arendt-Nielsen, 2012; Giesler & Liebeskind, 1976). Thus, we interpreted our results as evidence that the different types of internal pain caused by gallamine and hypertonic saline can function as a US that supports CTA learning.

Another type of internal pain is caused by lactose malabsorption (e.g., Deng, Misselwitz, Dai and Fox, 2015; Johnson, Kretchmer & Simoons, 1974). Lactose, a sweet-tasting disaccharide that is found in mammalian milk, cannot be absorbed unless it is first hydrolyzed into its monosaccharide elements (galactose and glucose) by the enzyme lactase. This enzyme is present in the intestinal tract in maximal quantities at birth through weaning but thereafter levels show a steep decline in both rats and humans (Büller, Kothe, Goldman, Grubman, Sasak, Matsudaira, Montgomery & Grand, 1990). In adults, the hallmarks of lactose intolerance are abdominal distention and pain (Saavedra & Perman, 1989). Unabsorbed lactose can also cause bloating, borborygmus and diarrhea. Furthermore, there is evidence that galactose also has aversive post-ingestive consequences in adult rats (e.g., Sclafani, Fanizza, & Azzara, 1999; Sclafani & Williams, 1999). Thus, even digested lactose can serve as an aversive US. This leads to our experimental question: Does lactose malabsorption in the adult rat induce CTA or TAL?

Only one study has investigated this issue in experimentally naïve rats¹. Pelchat et al. (1983) concluded that lactose-induced taste suppression should be interpreted as TAL. However, some design issues undermine confidence in this conclusion. The claim about the absence of a downshift in palatability was based on a taste reactivity analysis of responses,

or absence thereof, elicited by the CS following two conditioning trials. In the standard taste reactivity procedure (Grill, 1985; Grill & Berridge, 1985; Grill & Norgren, 1978), the taste stimulus is infused directly into the mouth via an intraoral catheter. The evoked orofacial and somatic responses can be classified as either ingestive or aversive. Pelchat et al. used an unconventional taste reactivity procedure in which the experimental animals could voluntarily consume a solution of 40% lactose on the two conditioning trials (i.e., lactose served as the CS and the US). This design choice allows for the monitoring of voluntary intake and the recording of taste reactivity responses. However, use of the hybrid procedure has several problematic consequences. First, the experimenter relinquishes control of US dose when amount consumed by each subject is the determining factor (on the first conditioning trial of the Pelchat et al. experiment, lactose intake ranged from 0.3 ml to 15.0 ml). Second, licking and taste reactivity are competing behavioral responses, which presumably limit the opportunity for the observation of ingestive taste reactivity responses. Third, when voluntary intake is low (or zero) there are fewer (or no) opportunities for the occurrence of taste reactivity responses producing a floor effect in the detection of aversive taste reactivity responses. Finally, it is an assumption that the taste reactivity repertoire is identical in all respects during voluntary drinking and intraoral infusions.

These concerns encouraged a re-examination of the nature of the taste learning supported by lactose malabsorption. We used lick pattern analysis because intake and palatability can be assessed simultaneously with this methodology. If lactose malabsorption supports TAL there should be a decrease in total licks, but no change in lick cluster size or initial lick rate in the experimental subjects (Group Lactose) relative to the control rats (Group Control). On the other hand, if lactose malabsorption supports CTA we expect to find a reduction in total licks, lick cluster size, and initial lick rate in Group Lactose compared to Group Control. To afford comparability with our previous research (and to avoid one of the issues with the Pelchat et al. [1983] design), we employed a procedure in which the CS and US were separate events. Thus, we used 0.1% saccharin as the CS and 20% lactose as the US (5.7 g/kg body weight administered at room temperature via a gastric catheter). To minimize the influence of stomach distension on performance, CS intake on the two conditioning trials was capped to a maximum of 2000 licks (~10 ml). Prior work reveals that clusters size is prone to increased variance when intake is capped (Lin et al., 2013). Therefore, as in that earlier research, two CS only test trials with 15-min unlimited access were scheduled to provide a more complete picture of the palatability of the taste CS. Finally, to ensure equal exposure to the US, the rats in the control group were given an intragastric infusion of lactose 24 h after the experimental rats received each CS-US pairing.

2. Materials and method

2.1. Subjects

Twenty male Sprague-Dawley rats weighing approximately 300 g were obtained from Charles River Laboratories (Wilmington, VT). They were individually housed in polycarbonate cages (Ancare, Bellmore, NY) in a room with a 12:12 h light:dark cycle that

¹Other studies have examined similar issues in non-naïve rats (i.e., Simbayi 1987; Simbayi, Boakes, & Burton, 1986; see Reilly & Bornova, 2005 for a discussion of the interpretational issues surrounding these results).

was maintained at ~70°F. The rats were given *ad libitum* access to food (Harlan 2018; Harlan Laboratories, Madison, WI) and tap water except as noted in the Procedure section below. The University of Illinois at Chicago Animal Care and Use Committee approved all procedures. Rats were treated according to guidelines provided by the American Psychological Association (2012) and the National Institutes of Health (2011).

2.2. Surgery

The rats were allowed to habituate to the facility for a minimum of 5 days prior to surgery when they were anesthetized with a mixture of ketamine (100 mg/kg, ip) and xylazine (10 mg/kg, ip) and fitted with a gastric catheter (e.g., Davis & Campbell, 1975; Touzani & Sclafani, 2001). Briefly, sterile tubing (OD: 0.065 in; Braintree Scientific Inc., Braintree, MA) was inserted into the fundus of the stomach and secured with sutures. The tubing was routed subcutaneously to the mid-scapular region where it was attached to a dorsal port (Plastics One, Roanoke, VA) and secured with wound clips. Catheters were filled with sterile saline and closed with dust caps (Plastics One). Following surgery animals were treated with analgesics (meloxicam, 1 mg/kg, sc) and antibiotics (enrofloxacin, 23 mg/kg, sc) once daily for a total of 3 days. Catheters were flushed with ~1 ml of room temperature water daily to ensure patency.

2.3. Apparatus

Eight identical drinking chambers (Med Associates, St. Albans, VT) were used to collect lick data with a 10 ms temporal resolution. As described in detail previously (e.g., Arthurs et al., 2012), each chamber was located inside a sound-attenuating cubicle and contained a single retractable sipper tube that could be accessed via an oval -shaped hole (1.3 cm × 2.6 cm) in the middle of the right-side wall. To prevent constant contact during drinking, in the extended position the tip of the sipper tube was ~3 mm outside the center of the access hole. A computer in an adjoining room running Med-PC software (Med Associates) and programs written in MedState Notation controlled chamber operation and data collection.

2.4. Procedure

Subjects were adapted to a deprivation schedule that allowed 15 min access to water (capped at 2000 licks) each morning in the drinking chamber and 15 min uncapped access to water in the home cage each afternoon. When the dependent measures were stable across three consecutive morning water sessions, the rats were counterbalanced into one of two groups ($n = 10/\text{group}$) in terms of their performance and the experiment began the next day. Conditioning trials occurred in three-day cycles; water was always available for 15 min each afternoon in the home cage. On Day 1, 0.1% saccharin (the CS) was substituted for water in the drinking chambers and followed, 5 min after removal of the rat from the drinking chamber, by an intragastric infusion (~1 min; e.g., Lucas & Sclafani, 1989), via a syringe connected to the intragastric cannula, of either lactose (5.7 g/kg; delivered via a 20% w/v lactose solution at 2.85 ml/100 g bodyweight) Group Lactose (bodyweight 451.0 ± 13.1 g) or an equivalent volume of water in Group Control (bodyweight 444.0 ± 9.4 g). Two hours after morning water access on Day 2, each rat in Group Lactose was given an intragastric infusion of water whereas the rats in Group Control were infused with lactose. Day 3 was a recovery day on which all rats were given 15-min capped access to water in the drinking

chamber and no intragastric infusion. On Days 4 – 6, a second conditioning cycle was administered. Beginning on Day 7, two CS only test trials were administered. The test trials were identical to conditioning trials, except all rats were given 15-min unlimited access to the CS each morning and there were no intragastric infusions.

2.5. Dependent Variables

The three dependent variables were: total licks, lick cluster size, and initial lick rate. Using our standard criteria (e.g., Arthurs et al., 2012), lick cluster size was defined as a run of licks separated by pauses (inter-lick intervals) of less than 500 ms and initial lick rate was defined as the total number of licks in the 3-min that followed the first lick.

2.6. Data Analysis

Each dependent variable was analyzed with a mixed design (Group x Trial) analysis of variance (ANOVA) with effect size reported as partial eta-squared (η_p^2). As necessary, significant main effects and interactions were followed up by post-hoc comparisons, simple main effects adopting a pooled error term from the overall ANOVA. All statistical analyses were conducted using *Statistica* software (Version 13; Dell Inc., 2015).

3. Results

Four animals were excluded from the study because of blockages in gastric catheters, reducing the number of subjects in each group to eight.

Water consumption in the drinking boxes took 13 days to stabilize across each dependent measure. Table 1 shows the performance of the two groups over the final three days of baseline water training. For each of the three measures (total licks, lick cluster size and initial lick rate) there were no significant main effects or interactions (all $ps > .05$).

The performance of each group during the two conditioning trials, where intake was capped at 2000 licks, is summarized in Figure 1. It will be evident from inspection of the figure that one CS-US pairing was sufficient for lactose malabsorption to cause a reduction in total licks, cluster size and initial lick rate. That is, compared to relatively high stable performance in the Control group Lactose animals exhibited a reduction from Trial 1 to Trial 2 across each dependent measure. For total licks (see Figure 1A) there was a significant Group x Trial interaction, $F(1,14) = 6.15$, $p < .05$, $\eta_p^2 = .305$, as well as significant main effects of Group, $F(1,14) = 8.0$, $p < .05$, $\eta_p^2 = .364$ and Trial, $F(1,14) = 13.04$, $p = .001$, $\eta_p^2 = .482$. Planned comparisons of the interaction found no group differences on Trial 1 ($p > .05$) but revealed that, relative to Group Control, Group Lactose made significantly fewer licks on Trial 2 ($p < .05$). Analysis of the lick cluster size data (see Figure 1B) found a significant main effect of Trial, $F(1,14) = 8.38$, $p < .05$, $\eta_p^2 = .375$, but there was no main effect of Group ($F < 1$) and no significant Group x Trial interaction ($p > .05$). Notably, relative to the Control group (Trial 1, $M = 193.76$; Trial 2, $M = 102.32$) there was a much larger between trials numerical decrease in lick cluster size for the Lactose group (Trial 1, $M = 324.83$; Trial 2, $M = 17.43$). However, the interaction term failed to reach significance

likely due to the large degree of variability in the lick cluster size data. Finally, for initial lick rate (see Figure 1C) there was a significant Group x Trial interaction, $F(1,14) = 6.01$, $p < .05$, $\eta_p^2 = .300$, and significant main effect of Trial, $F(1,14) = 7.52$, $p < .05$, $\eta_p^2 = .349$; the main effect of Group was not significant, $F(1,14) = 3.97$, $p = .066$, $\eta_p^2 = .221$. Planned comparisons found no between-group differences on Trial 1 but revealed that initial lick rate was significantly lower in the Lactose group compared to the Control group on Trial 2 ($p < .05$).

Data from the two CS only test trials, where unlimited access was available for 15 min, are summarized for the two groups in Figure 2. It is immediately evident from inspection of the figure that lactose malabsorption caused a substantial reduction in both the intake and palatability of the associated saccharin CS, characterizations that were confirmed by statistical analyses. In terms of total licks (see Figure 2A) there were significant main effects of Group, $F(1,14) = 23.00$, $p < .05$, $\eta_p^2 = .622$, and Trial, $F(1,14) = 7.97$, $p < .05$, $\eta_p^2 = .363$, but the Group x Trial interaction was not significant ($F < 1$). For lick cluster size (see Figure 2B) there was a significant main effect of Group, $F(1,14) = 15.20$, $p < .05$, $\eta_p^2 = .521$, but neither the main effect of Trial ($F < 1$) nor the Group x Trial interaction ($F < 1$) was significant. The same pattern of significance was shown by initial lick rate (see Figure 2C): a significant main effect of Group, $F(1,14) = 24.07$, $p < .05$, $\eta_p^2 = .632$, but not significant main effect of Trial ($p > .30$) and no significant Group x Trial interaction ($F < 1$).

4. Discussion

Concerns about the experimental design as well as with the interpretation of the results reported by Pelchat et al. (1983) encouraged a re-examination of whether a lactose US supports TAL or CTA. The present results indicate that lactose malabsorption produced a conditioned suppression of both intake and palatability. As shown in Figure 1, comparable unconditioned levels of performance were evident in the Control group and the Lactose group on Trial 1. However, after a single saccharin-lactose pairing, there was a large numerical decrease in total licks, initial lick rate, and lick cluster size in the Lactose group relative to their performance on Trial 1. There was also a clear Trial 2 difference between the Control and Lactose groups for total licks and initial lick rate. As noted in the Introduction, cluster size is susceptible to high levels of variance when intake is limited, so it was not surprising when a similar high level of variance, which obscured statistical analysis, was found in the present experiment. As expected, the lactose-induced suppression of intake and palatability was more clearly displayed during the test trials (see Figure 2) in which there were highly significant differences between the Control and Lactose groups on all three dependent measures. Thus, the present results provide clear evidence that lactose malabsorption supports CTA, not TAL.

Pelchat et al. (1983) used a 40% solution of lactose as the US. To keep the lactose in suspension during the conditioning and test trials, the solution was served to the rats at 35°C. Furthermore, to prevent the temperature of the lactose from serving as a cue the daily water was also warmed to 35°C in that experiment. In part to avoid this design complication, in the present experiment we used intragastric infusions to obtain the desired dosing while using a

lactose concentration that was stable at room temperature (Machado, Coutinho, & Macedo, 2000; Roos, 2002). We speculate that on the first conditioning trial that the relatively large volume of water infusion received by the control animals may have caused some mildly unpleasant stomach distension, resulting in the reduction of cluster size shown by the Control group on the second acquisition trial. This downshift in cluster size was also evident on the first test trial but, as noted above, there were highly significant differences between the Control and Lactose group on this dependent measure.

As reported in the present article, the abdominal pain caused by lactose malabsorption is an effective US that supports CTA acquisition. Using the same approach (lick pattern analysis in a voluntary intake procedure), we have also found that the muscular pain induced with gallamine and the visceral pain induced by hypertonic saline are each effective USs that support CTA acquisition (Lin et al., 2013). Similarly, we have recently found that anesthesia-inducing drugs (ketamine/xylazine and sodium pentobarbital) can serve as USs to support CTA learning (Lin et al., 2017a). Finally, we have discovered that drugs of abuse (e.g., amphetamine and morphine), at dose that are rewarding in other tasks (i.e., place - preference learning and self-administration tasks [e.g., Cappell & LeBlanc, 1971; Cappell, LeBlanc, & Endrenyi, 1973; Hunt & Amit, 1987; Parker, Limebeer, & Rana, 2009; Schuster & Thompson, 1969]), are capable of supporting CTA acquisition (e.g., Arthurs et al., 2012; Arthurs & Reilly, 2013; Lin, Arthurs, Amodeo & Reilly, 2012; for reviews see Lin et al., 2014, 2017b). All these findings, particularly those with drugs of abuse, are at odds with the conclusions derived from research that employed the taste reactivity test to determine palatability of the taste CS. Specifically, with drug of abuse USs it is reported that there is no conditioned downshift in palatability of the associated taste CS consequent to contingent taste-drug pairings (e.g., Parker, 1988, 1991; Parker & Carvell, 1986) and that drug of abuse USs support TAL (for reviews see Parker, 1991, 1995, 2003; Parker et al., 2009).

It may be tempting to believe that the different methods of palatability assessment—lick pattern analysis and the taste reactivity test—yield different results in the analysis of CTA and TAL. This, however, is not the case. Indeed, we believe that these methods are equally valid. Rather, the issue is entirely based on one's theoretical stance on the definition of palatability—whether palatability is a one- or two-dimensional construct. Lick pattern analysis is inherently a one-dimensional account, ranging along a continuum from highly positive (i.e., large cluster size and fast initial rates of responding) to highly negative (i.e., no responding). The two categories of taste reactivity responses (ingestive and aversive) can be viewed as supporting either a one- or a two-dimensional account. For the one-dimensional account (e.g., Breslin, Spector, & Grill, 1992; Spector, Breslin, & Grill, 1988) palatability varies from high levels of ingestive responses to high levels of aversive responses with a low level of either type of response in the center of the continuum. By this analysis, a conditioned reduction in the frequency of ingestive responses provides evidence of mild-to-moderate CTAs. On the other hand, a two-dimensional account of palatability (Berridge & Grill, 1983, 1984; Parker, 1991; 1995; 2003) views each category of taste reactivity responses (ingestive and aversive) as representing independent dimensions. By this analysis, the occurrence of aversive rejection responses (e.g., gaping—indicative of retching or vomiting in the non-emetic rat; Travers & Norgren, 1986) is the sole indication that a solution is aversive and disgusting. Because drug of abuse USs are typically used at low-

moderate doses they do not support the development of conditioned gaping responses, although they do cause a conditioned reduction in ingestive responding (e.g., Parker, 1991; 1995; 2003). Thus, using a gaping-dependent definition of aversion leads to the conclusions that drug of abuse USs induce TAL not CTA.

Although using the occurrence of conditioned gapes as the defining characteristic of CTA may be appealing, this interpretation carries some problematic consequences. For example, this definition transforms CTA learning into a binary phenomenon: that is, in the absence of gaping there is no CTA and the detection of a significant number of gapes defines the presence of a CTA. The problem with this definition becomes apparent once other species are considered. For instance, primates display an additional aversive taste reactivity response—midface grimacing—that does not appear in the rat (Steiner, Glaser, Hawilo, & Berridge, 2001). On the spectrum of taste reactivity responses, midface grimacing occurs in response to stimuli that are mildly aversive but do not necessarily produce gaping. Therefore, one could define CTA in primates based on a significant increase in midface grimaces, which would still be a binary definition of CTA, but would be a more sensitive definition of CTA, in terms of taste reactivity responses, than is possible in the rat.

Recent work supports the idea that CTAs induced with LiCl or hypertonic saline involve a decrease in both intake and palatability, but that these dissimilar CTA-inducing USs may result in some differential behavioral responses (Dwyer, Gasalla, Bura, & Lopez, 2017). Dwyer and colleagues compared CTAs induced by either LiCl or hypertonic saline as assessed by taste reactivity and voluntary intake, while monitoring locomotor activity (to determine fear-induced freezing) during the taste reactivity trials. They report that hypertonic saline can induce a CTA (i.e., significant decreases in intake, cluster size and ingestive taste reactivity; no changes in aversive taste reactivity were found) while also causing an increase in time freezing. Conversely, LiCl induced a CTA (i.e., decreases in intake, cluster size, and ingestive taste reactivity as well as increased aversive taste reactivity) while also supporting a significant elevation in freezing (to a level ~50% that of the hypertonic saline US in Experiment 3), indicating that conditioned fear contributes to LiCl-induced taste learning.

It is difficult, however, to rule out that these two USs (hypertonic saline and LiCl) may simply have induced CTAs of different magnitudes. That is, once voluntary intake is completely suppressed there is the question of floor effects causing a loss of contact with the strength of a CTA in terms of all dependent measures except behavioral responses to involuntary intraoral infusions (i.e., gaping), which is a completely different scale from voluntary intake. Also, as in the Pelchat et al. (1983) study, certain design choices may not have been optimal, including sequential rather than concurrent lick and taste reactivity analysis as well as context changes between conditioning and test trials, which may influence behavior during extinction. Nonetheless, the results of Dwyer et al. (2017) raise the possibility that, while many stimuli can produce CTA, nausea-inducing stimuli may have a particularly profound influence on conditioned gaping. Conversely, painful stimuli may have an equally profound effect on measures of fear-like behavior, such as freezing. Moreover, it is clear that this relationship is non-orthogonal as LiCl can cause an increase in conditioned freezing and hypertonic saline can reduce taste palatability. Notably, while there

was no significant increase in aversive taste reactivity with the doses of hypertonic saline tested in the Dwyer et al. study this does not preclude such an effect if higher doses were to be examined. Bearing these caveats in mind, these findings would seem to fit with the present results, as well as our theory that CTA is a broadly-tuned defense mechanism inherently prone to false positives (e.g., Lin et al., 2014; 2017a).

In sum, the present results, obtained using lick pattern analysis to determine conditioned changes in taste palatability, add lactose malabsorption to a growing list of atypical USs that produce CTAs, which include drugs of abuse (e.g., amphetamine, morphine), anesthetic drugs (e.g., ketamine/xylazine, pentobarbital) and internal pain (e.g., hypertonic saline, gallamine). Thus, CTAs can be induced by a wider range of USs than those that are traditionally known to cause gastrointestinal malaise, illness, nausea or sickness (e.g., poisons, toxins, chemotherapy drugs, radiation, vestibular disorientation). Refining our understanding of the distinction between CTA and TAL is not only of great theoretical importance but will also guide analysis of the neural underpinnings of CTA.

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References

- American Psychological Association. Guidelines for ethical conduct in the care and use of nonhuman animals in research. 2012.
- Arthurs J, Lin JY, Amodeo LR, Reilly S. Reduced palatability in drug-induced taste aversion: II. Aversive and rewarding unconditioned stimuli. *Behavioral Neuroscience*. 2012; 126:433–444. [PubMed: 22409482]
- Arthurs J, Reilly S. Role of the gustatory thalamus in taste learning. *Behavioural Brain Research*. 2013; 250:9–17. [PubMed: 23644184]
- Baird JP, St John SJ, Nguyen EAN. Temporal and qualitative dynamics of conditioned taste aversion processing: combined generalization testing and licking microstructure analysis. *Behavioral Neuroscience*. 2005; 119:983–1003. [PubMed: 16187827]
- Barker, LM, Best, MR., Domjan, M., editors. *Learning Mechanisms in Food Selection*. Waco, TX: Baylor University Press; 1977.
- Berridge KC, Grill HJ. Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rat. *Behavioral Neuroscience*. 1983; 97:563–573. [PubMed: 6615632]
- Berridge KC, Grill HJ. Isohedonic tastes support a two-dimensional hypothesis of palatability. *Appetite*. 1984; 5:221–231. [PubMed: 6524918]
- Braveman, NS., Bronstein, P., editors. *Experimental Assessments and Clinical Applications of Conditioned Food Aversions*. New York Academy of Sciences; 1985.
- Breslin PA, Spector AC, Grill HJ. A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium chloride pairings: a unidimensional account of palatability. *Behavioral Neuroscience*. 1992; 106:820–836. [PubMed: 1332732]
- Brett, LP. Unpublished doctoral dissertation. University of California; Los Angeles: 1977. Experimental extensions of the cue-consequence aversive conditioning paradigm.
- Büller HA, Kothe MJ, Goldman DA, Grubman SA, Sasak WV, Matsudaira PT, ... Grand RJ. Coordinate expression of lactase-phlorizin hydrolase mRNA and enzyme levels in rat intestine during development. *Journal of Biological Chemistry*. 1990; 265:6978–6983. [PubMed: 1691182]

- Cull-Candy SG, Miledi R. Block of glutamate-activated synaptic channels by curare and gallamine. *Proceedings of the Royal Society of London B: Biological Sciences*. 1983; 218:111–118. [PubMed: 6135207]
- Davis JD. The microstructure of ingestive behavior. *Annals of the New York Academy of Sciences*. 1989; 575:106–121. [PubMed: 2699182]
- Davis JD. Deterministic and probabilistic control of the behavior of rats ingesting liquid diets. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 1996; 270:793–800.
- Davis JD. A model for the control of ingestion — 20 years later. *Progress in Psychobiology and Physiological Psychology*. 1998; 17:127–173.
- Davis, JD., Campbell, CS. Chronic intrajugular, intraportal, gastric, and duodenal cannulae for the rat. In: Singh, D., Avery, DD., editors. *Physiological Techniques in Behavioral Research*. Monterey: Brooks/Cole Publishing; 1975. p. 163-177.
- Davis JD, Perez MC. Food deprivation-and palatability-induced microstructural changes in ingestive behavior. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 1993; 264:97–103.
- Davis JD, Smith GP. Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. *Behavioral Neuroscience*. 1992; 106:217–228. [PubMed: 1554433]
- Dell Inc. Dell Statistica (data analysis software system). Version 13. Software.dell.com; 2015.
- Deng Y, Misselwitz B, Dai N, Fox M. Lactose intolerance in adults: biological mechanism and dietary management. *Nutrients*. 2015; 7:8020–8035. [PubMed: 26393648]
- Drewes AM, Babenko L, Birket-Smith L, Funch-Jensen P, Arendt-Nielsen L. Induction of non-painful and painful intestinal sensations by hypertonic saline: a new human experimental model. *European Journal of Pain*. 2003; 7:81–91. [PubMed: 12527321]
- Dwyer DM. Licking and liking: The assessment of hedonic responses in rodents. *Quarterly Journal of Experimental Psychology*. 2012; 65:371–394.
- Dwyer DM, Boakes RA, Hayward AJ. Reduced palatability in lithium-and activity-based, but not in amphetamine-based, taste aversion learning. *Behavioral Neuroscience*. 2008; 122:1051–1060. [PubMed: 18823162]
- Dwyer DM, Gasalla P, Bura S, Lopez M. Flavors paired with internal pain or with nausea elicit divergent types of hedonic responses. *Behavioral Neuroscience*. 2017; 131:235–248. [PubMed: 28471224]
- Garcia J, Koelling RA. Relation of cue to consequence in avoidance learning. *Psychonomic Science*. 1966; 4:123–124.
- Garcia J, Kovner R, Green KF. Cue properties vs palatability of flavors in avoidance learning. *Psychonomic Science*. 1970; 20:313–314.
- Giesler GJ, Liebeskind JC. Inhibition of visceral pain by electrical stimulation of the periaqueductal gray matter. *Pain*. 1976; 2:43–48. [PubMed: 1028018]
- Grill HJ. Introduction: Physiological mechanisms in conditioned taste aversions. *Annals of the New York Academy of Sciences*. 1985; 44(3):67–88.
- Grill HJ, Berridge KC. Taste reactivity as a measure of the neural control of palatability. *Progress in Psychobiology and Physiological Psychology*. 1985; 11:1–61.
- Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research*. 1978; 143:263–279. [PubMed: 630409]
- Higgs S, Cooper SJ. Effects of the benzodiazepine receptor inverse agonist Ro 15–4513 on the ingestion of sucrose and sodium saccharin solutions: A microstructural analysis of licking behavior. *Behavioral Neuroscience*. 1996; 110:559–566. [PubMed: 8889001]
- Ionescu E, Burešová O. Failure to elicit conditioned taste aversion by severe poisoning. *Pharmacology Biochemistry and Behavior*. 1977; 6:251–254.
- Johnson, JD., Kretchmer, N., Simoons, FJ. Lactose malabsorption; its biology and history. In: Schulman, I., editor. *Advances in Pediatrics*. Vol. 21. Chicago: Yearbook Medical Publishers; 1974. p. 197-237.

- Katsuura Y, Heckmann JA, Taha SA. μ -Opioid receptor stimulation in the nucleus accumbens elevates fatty tastant intake by increasing palatability and suppressing satiety signals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2011; 301:244–254.
- Kent WD, Cross-Mellor SK, Kavaliers M, Ossenkopp KP. Acute effects of corticosterone on LiCl-induced rapid gustatory conditioning in rats: A microstructural analysis of licking patterns. *Behavioural Brain Research*. 2002; 136:143–150. [PubMed: 12385799]
- Lett BT. The painlike effect of gallamine and naloxone differs from sickness induced by lithium chloride. *Behavioral Neuroscience*. 1985; 99:145–150. [PubMed: 2994689]
- Lin JY, Arthurs J, Amodeo LR, Reilly S. Reduced palatability in drug-induced taste aversion: I. Variations in the initial value of the conditioned stimulus. *Behavioral Neuroscience*. 2012; 126:423–432. [PubMed: 22428901]
- Lin JY, Arthurs J, Reilly S. Reduced palatability in pain-induced conditioned taste aversions. *Physiology & Behavior*. 2013; 119:79–85. [PubMed: 23769688]
- Lin JY, Arthurs J, Reilly S. Conditioned taste aversion, drugs of abuse and palatability. *Neuroscience & Biobehavioral Reviews*. 2014; 45:28–45. [PubMed: 24813806]
- Lin JY, Arthurs J, Reilly S. Anesthesia-inducing drugs also induce conditioned taste aversions. *Physiology & Behavior*. 2017a; 177:247–251. [PubMed: 28499795]
- Lin JY, Arthurs J, Reilly S. Conditioned taste aversions: From poisons to pain to drugs of abuse. *Psychonomic Bulletin & Review*. 2017b; 24:335–351. [PubMed: 27301407]
- Lucas F, Sclafani A. Flavor preferences conditioned by intragastric fat infusions in rats. *Physiology & Behavior*. 1989; 46:403–412. [PubMed: 2623061]
- Machado JJ, Coutinho JA, Macedo EA. Solid–liquid equilibrium of α -lactose in ethanol/water. *Fluid Phase Equilibria*. 2000; 173:121–134.
- Milgram, NW. Krames, L., Alloway, T., editors. *Food Aversion Learning*. New York: Plenum Press; 1977.
- National Institutes of Health. *Guide for the care and use of laboratory animals*. Washington DC: National Academy Press; 2011.
- Overduin J, Figlewicz DP, Bennett-Jay J, Kittleson S, Cummings DE. Ghrelin increases the motivation to eat, but does not alter food palatability. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2012; 30(3):259–269.
- Parker LA. Positively reinforcing drugs may produce a different kind of CTA than drugs which are not positively reinforcing. *Learning & Motivation*. 1988; 19:207–220.
- Parker LA. Taste reactivity responses elicited by reinforcing drugs: a dose-response analysis. *Behavioral Neuroscience*. 1991; 105:955–964. [PubMed: 1777108]
- Parker LA. Rewarding drugs produce taste avoidance, but not taste aversion. *Neuroscience & Biobehavioral Reviews*. 1995; 19:143–151. [PubMed: 7770194]
- Parker LA. Taste avoidance and taste aversion: evidence for two different processes. *Animal Learning & Behavior*. 2003; 31:165–172.
- Parker LA, Carvell T. Orofacial and somatic responses elicited by lithium-, nicotine- and amphetamine-paired sucrose solution. *Pharmacology, Biochemistry & Behavior*. 1986; 24:883–887.
- Parker, LA., Limebeer, CL., Rana, SA. Conditioned disgust, but not conditioned taste avoidance, may reflect conditioned nausea in rats. In: Reilly, S., Schachtman, TR., editors. *Conditioned Taste Aversion: Behavioral and Neural Processes*. Oxford University Press; Oxford, UK: 2009. p. 92–113.
- Pelchat ML, Grill HJ, Rozin P, Jacobs J. Quality of acquired responses to tastes by *Rattus norvegicus* depends on type of associated discomfort. *Journal of Comparative Psychology*. 1983; 97:140–153. [PubMed: 6307586]
- Reilly S, Bornova MA. Conditioned taste aversion and amygdala lesions in the rat: a critical review. *Neuroscience & Biobehavioral Reviews*. 2005; 29:1067–1088. [PubMed: 15893375]
- Reilly, S., Schachtman, TR., editors. *Conditioned Taste Aversion: Behavioral and Neural Processes*. Oxford University Press; Oxford UK: 2009.
- Roos YH. Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait*. 2002; 82(4):475–484.

- Sakai N, Yamamoto T. Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various USs. *Neuroreport*. 1997; 8:2215–2220. [PubMed: 9243614]
- Saavedra M, Perman A. Current concepts in lactose malabsorption and intolerance. *Annual Review of Nutrition*. 1989; 9:475–502.
- Sclafani A, Fanizza LJ, Azzara AV. Conditioned flavor avoidance, preference, and indifference produced by intragastric infusions of galactose, glucose, and fructose in rats. *Physiology & Behavior*. 1999; 67:227–234. [PubMed: 10477054]
- Sclafani A, Williams DL. Galactose consumption induces conditioned flavor avoidance in rats. *Journal of Nutrition*. 1999; 129:1737–1741. [PubMed: 10460213]
- Simbayi LC, Boakes RA, Burton MJ. Effects of basolateral amygdala lesions on taste aversions produced by lactose and lithium chloride in the rat. *Behavioral Neuroscience*. 1986; 100:455–465. [PubMed: 3017373]
- Simbayi LC. Effects of anterior basolateral amygdala lesions on taste aversions produced by high and low oral doses of LiCl and lactose in the rat. *Behavioural Brain Research*. 1987; 25:131–142. [PubMed: 2823849]
- Spector AC, Breslin P, Grill HJ. Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste-visceral associations. *Behavioral Neuroscience*. 1988; 102:942–952. [PubMed: 2850815]
- Spector AC, Klumpp PA, Kaplan JM. Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. *Behavioral Neuroscience*. 1998; 112:678–694. [PubMed: 9676983]
- Spector AC, Smith JC. A detailed analysis of sucrose drinking in the rat. *Physiology & Behavior*. 1984; 33:127–136. [PubMed: 6505048]
- Spector AC, St John SJ. Role of taste in the microstructure of quinine ingestion by rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 1998; 274:1687–1703.
- Steiner JE, Glaser D, Hawilo ME, Berridge KC. Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neuroscience & Biobehavioral Reviews*. 2001; 25:53–74. [PubMed: 11166078]
- Touzani K, Sclafani A. Lateral hypothalamic lesions impair flavour-nutrient and flavour-toxin trace learning in rats. *European Journal of Neuroscience*. 2002; 16:2425–2433. [PubMed: 12492437]
- Travers JB, Norgren R. Electromyographic analysis of the ingestion and rejection of sapid stimuli in the rat. *Behavioral Neuroscience*. 1986; 100:544–555. [PubMed: 3741605]

Highlight

- TAL involves a learned reduction in intake only
- 2. CTA involves learned reductions in intake and palatability
- 3. Lactose malabsorption in rats induces a CTA, not, as previously thought, a TAL
- 4. Current results indicate a broader tuning of CTA than commonly acknowledged

Fig. 1.

Mean ($\pm$ SE) conditioned stimulus-directed performance during the two conditioning trials. Rats in Group Control received 0.1% saccharin followed by an intragastric infusion of water, whereas those in Group Lactose received saccharin followed by an intragastric infusion of lactose. During each trial rats were allowed 15-min to make a maximum of 2000 licks. A: Total licks; B: lick cluster size; C: initial lick rate. Note: the large variance in lick cluster size on Trial 1 necessitating an expanded ordinate axis relative to Figure 2.

Fig. 2. Mean ($\pm$ SE) conditioned stimulus-directed performance during the two uncapped 15-min saccharin only test trials in the control (Group Control) and experimental (Group Lactose) rats. A: Total licks; B: lick cluster size; C: initial lick rate. Note: relative to Figure 1, a different scale has been used for the y-axis in Panel B due to less variance in the data.

Table 1

Water consumption (mean $\pm$ SE) on each of the final three morning baseline trials was characterized using three dependent measures: total licks, cluster size, and initial lick rate (licks in the 3 min that followed the first lick).

Group	Day	Total licks	Cluster size	Initial lick rate
Control	-1	1945.25 $\pm$ 43.35	189.72 $\pm$ 49.83	1060.63 $\pm$ 29.65
	-2	1994.88 $\pm$ 5.27	125.32 $\pm$ 26.90	1029.38 $\pm$ 31.42
	-3	1984.88 $\pm$ 15.13	168.17 $\pm$ 35.40	1053.75 $\pm$ 37.08
Lactose	-1	2000.00 $\pm$ 0.00	276.77 $\pm$ 70.93	1086.00 $\pm$ 33.05
	-2	2000.00 $\pm$ 0.00	279.64 $\pm$ 105.84	1059.88 $\pm$ 28.24
	-3	2000.00 $\pm$ 0.00	248.68 $\pm$ 51.97	1096.38 $\pm$ 27.38
Control	-1	1945.25 $\pm$ 43.35	189.72 $\pm$ 49.83	1060.63 $\pm$ 29.65
	-2	1994.88 $\pm$ 5.27	125.32 $\pm$ 26.90	1029.38 $\pm$ 31.42
	-3	1984.88 $\pm$ 15.13	168.17 $\pm$ 35.40	1053.75 $\pm$ 37.08
Lactose	-1	2000.00 $\pm$ 0.00	276.77 $\pm$ 70.93	1086.00 $\pm$ 33.05
	-2	2000.00 $\pm$ 0.00	279.64 $\pm$ 105.84	1059.88 $\pm$ 28.24
	-3	2000.00 $\pm$ 0.00	248.68 $\pm$ 51.97	1096.38 $\pm$ 27.38