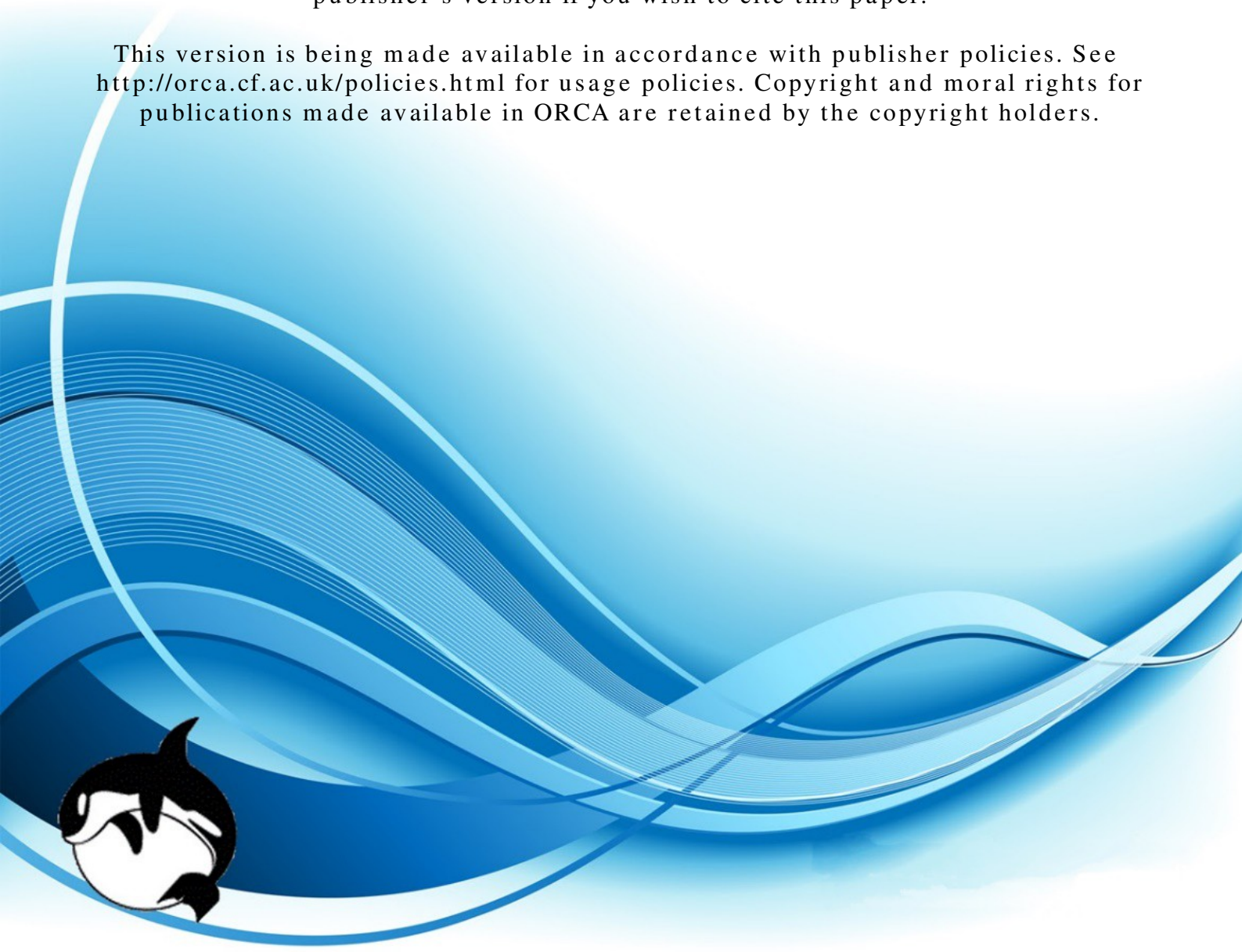

Flavors paired with internal pain or with nausea elicit divergent types of hedonic responses.

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Abstract

Pairing a taste with either internal pain (e.g. from hypertonic saline injection) or nausea (e.g. from LiCl administration) will reduce subsequent consumption of that taste. Here we examine the responses to a taste paired with either hypertonic saline or LiCl using the analysis of licking microstructure (mean lick cluster size: Experiments 1-3), taste reactivity (examining the distribution of appetitive and aversive orofacial responses: Experiments 2-3), and immobility (as a measure of fear: Experiments 2-3). At both high (10ml/kg 0.15M LiCl, 10ml/kg 1.5M NaCl) and low dose levels (2ml/kg 0.15M LiCl, 4ml/kg 1.5M NaCl), pairing a taste with either LiCl-induced nausea or internal pain produced by hypertonic NaCl caused reductions in voluntary consumption, in appetitive taste reactivity responses, and in lick cluster size. However, only pairing with LiCl resulted in conditioned aversive taste reactivity responses to the taste. In contrast, pairing with hypertonic NaCl resulted in the taste eliciting higher levels of immobility (reflecting fear) than did pairing the taste with LiCl. The clearly dissociable effects of LiCl and hypertonic saline on aversive taste reactivity and fear responses, despite equivalent effects on consumption, demonstrates selective conditioning effects between internal pain and nausea.

Keywords: CTA, conditioned nausea, internal pain, taste reactivity, licking analysis, rats

1. **Introduction**

Although rats are incapable of vomiting, they readily learn to avoid foods paired with toxins that have previously caused them gastrointestinal malaise by acting on the emetic system of the midbrain and brainstem (Garcia, Hankins, & Rusiniak, 1974). This phenomenon is termed conditioned taste aversion (CTA) and potentially represents a key behavioral mechanism for toxin avoidance as well as providing a useful model for the study of anticipatory nausea in chemotherapy (Domjan, 1980; Garcia, Kimmeldorf, & Koelling, 1955; Garcia & Koelling, 1967; Parker, 2014; Reilly & Schachtman, 2009). Moreover, pairing a novel taste with emesis not only results in a reduction in consumption of that taste, but also produces a reduction in its palatability that can be revealed through a range of techniques (for reviews see, Lin, Arthurs, & Reilly, 2014; Parker, Rana, & Limebeer, 2008). However, emetic treatments are not alone in producing a reduction in consumption: pairing tastes with a wide variety of other events, including pain produced by footshock or injection of hypertonic saline, as well as the administration of many drugs of abuse, reliably produces dramatic reductions in voluntary consumption (e.g., Arthurs, Lin, Amodeo, & Reilly, 2012; Dwyer, Boakes, & Hayward, 2008; Parker, 1995a; Pelchat, Grill, Rozin, & Jacobs, 1983). Thus, one central issue in the analysis of taste aversion is whether emetic and non-emetic treatments operate through the same learning mechanisms. A key question in making this comparison is whether emetic and non-emetic treatments produce the same sorts of conditioned changes in taste palatability (compare, for example, Lin, Arthurs, & Reilly, 2016; with, Parker, 2003). We address this question by directly comparing the effects of an emetic treatment (injection of LiCl) with a non-emetic treatment (internal pain produced by injection of hypertonic NaCl). But before turning to the analysis of taste aversion mechanisms, it is important to consider the experimental methods used to assess palatability in rodents.

In the taste reactivity test (TR), 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 hedonic 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 et al. (1983) observed that while pairing sucrose with either LiCl-induced nausea or peripheral pain produced by footshock reduced the voluntary consumption of sucrose, only LiCl produced a change in TR responses (both an increase in aversive responses and a decrease in appetitive responses). Thus the assessment of TR behaviors provides information about why voluntary consumption has changed rather than merely assessing the size of that change.

An alternative approach for assessing palatability involves analyzing the microstructure of licking behavior (Davis, 1973, 1989; Dwyer, 2012; Lin, Amodeo, Arthurs, & Reilly, 2012). Rats ingest fluids in sustained runs of licks separated by pauses of varying length (clusters), and the mean number of licks per cluster (lick cluster size) is lawfully related to the nature of the solution ingested. For sweet-tasting solutions cluster size monotonically increases with concentration (e.g., Davis & Smith, 1992; Dwyer, 2008) while consumption shows an inverted U-shape relationship with increases in the concentration of the sweet solution (e.g., McCleary, 1953; Richter & Campbell, 1940): in effect, the more palatable the solution the larger the lick cluster size. In addition, there is a monotonic decrease in lick cluster size for quinine solutions as concentration increases (Hsiao & Fan, 1993; Spector & John, 1998) which parallels the increase in aversive TR responses elicited by this unpalatable bitter taste (Grill & Norgren, 1978; Parker & Lopez Jr, 1990). In the context of taste aversion learning, pairing a taste with LiCl has been reliably found to produce

a reduction in lick cluster size (e.g., Arthurs et al., 2012; Baird, St. John, & Nguyen, 2005; Dwyer et al., 2008).

Returning to conceptual issues, one influential analysis of taste aversion involves drawing a qualitative distinction between treatments that produce emesis and non-emetic treatments that produce the anticipation of a danger (Garcia, Kovner, & Green, 1970; Rozin & Kalat, 1971). This distinction, subsequently developed by Parker (2003) based on research using the TR method, suggests that taste aversions develop when a novel taste is followed by the state of nausea that causes both conditioned disgust, commonly observed as aversive TR responses, and decreased consumption of the taste. In addition, Parker also suggests that a different kind of process (taste avoidance learning, TAL) is engaged when a taste is followed by changes in the physiological state of the organism (changes in homeostasis) produced by non-emetic treatments and causes avoidance of the taste without disgust. That is, TAL reflects the anticipation of the negative consequences – a fear response. Parker suggests that rats display conditioned aversive responses exclusively to solutions paired with emetic drugs: for example, LiCl, apomorphine, or nicotine. In contrast, many treatments which do not produce nausea do not appear to produce conditioned aversive responses in the TR test: for example, many drugs of abuse, pain and lower intestinal discomfort (for extended reviews, see Parker, 2003, 2006, 2014). Interestingly, antiemetic treatments can block the expression of aversive TR responses without blocking the reduction in consumption seen with LiCl-paired tastes (Limebeer & Parker, 2000). In line with Garcia's classic description of the nature of the learning acquired when pain or emesis are employed as USs (Garcia et al., 1970), Parker's account suggests that conditioned nausea is the primary elicitor of changes in palatability, and therefore a necessary condition for developing a "true" conditioned taste aversion based on acquired disgust. Importantly, this approach is based on treating appetitive and aversive TR responses as reflecting two separate dimensions, with aversive responses

taken as an indication of disgust and appetitive responses as an indication of positive hedonic value.

Although Parker's analysis of the distinction between CTA and TAL has been influential, Reilly and colleagues have recently questioned this account (Lin, et al., 2014, 2016). They note that even though drugs of abuse may not cause aversive TR responses, they do produce significant reductions in appetitive TR responses (see, for example, Parker, 1991). In addition, using the analysis of the licking microstructure, Reilly's group examined the effect of pairing a novel taste with the administration of gallamine hydrochloride (10mg/kg), hypertonic saline (1.0 M) (Lin, Arthurs, & Reilly, 2013; Lin et al., 2014) and amphetamine (Lin, Arthurs, Amodeo, & Reilly, 2012). They found that these USs not only produced a reduction in consumption of the CS, but also decreased lick cluster size. In reviewing these results, Lin et al. (2014, 2016) argue that a reduction in appetitive responses or a reduction in lick cluster size might reflect a decrease in palatability that was less than that required to elicit strongly aversive taste reactivity responses such as gaping. Thus, internal pain or drugs of abuse might simply be producing lower levels of aversion than nausea produced by LiCl. That is, the difference in between drug/pain-induced and nausea-induced taste aversion would be quantitative rather than qualitative (i.e., a difference of degree vs a difference of kind). Reilly's analysis also includes a critique of the taste reactivity method per se and the details of the ways in which it has been applied. This includes the suggestion that TR responses should be viewed as a single continuum from highly positive (many appetitive TR responses) to highly negative (many aversive TR responses), and that mild aversions might be seen through the reduction in appetitive TR responses. This critique raises the possibility that focusing taste reactivity analysis mainly on strongly aversive responses such as gaping may leave the method insensitive to mild aversions.

The theoretical analyses of CTA provided by Parker and Reilly clearly and materially diverge – a difference emphasized by the reliance on different methodologies and different interpretations of TR responses. Moreover, much of the analysis of the similarities or differences in the effects of different USs in taste aversion learning rests on comparisons between separate experiments and between USs delivered in very different ways. For example, the comparison of peripheral pain from footshock with internally experienced emesis from LiCl injection is confounded with differences in the location and nature of delivery of these treatments. In contrast, hypertonic NaCl appears to be an ideal tool for producing internal pain as a comparison to nausea produced by LiCl. Hypertonic saline is thought to produce activation of pain fibers through elevating extra-cellular sodium concentration (and thus increasing sodium influx and depolarization) and human studies suggest the pain experienced is both local and referred (Staahl & Drewes, 2004). It is a well-established model of visceral pain (Giesler & Liebeskind, 1976; Ness & Gebhart, 1990) and matches the injection administration methods used for LiCl. Despite this, it has received no analysis using TR methods. Thus, the primary experimental aim for the current studies was to directly compare the effects of internal pain induced by hypertonic NaCl and nausea induced by LiCl using the TR test. However, before making this direct comparison, we first ensured that we could replicate the reduction in lick cluster size after taste-hypertonic NaCl pairing. We used one-trial conditioning (Experiment 1) with a high hypertonic NaCl dose to recapitulate the way in which LiCl can produce taste aversion learning with only a single training trial. Following this, we used the TR method to compare internal pain and nausea-based aversions. In particular, we compared the effect of both USs on TR with doses chosen to produce comparable levels of consumption change. This was done using both low doses LiCl and hypertonic NaCl in order to avoid floor effects (Experiment 2), and high US doses in order to avoid weak effects that might be undetectable by the TR test (Experiment 3).

2. Experiment 1

One notable feature of taste aversion learning based on LiCl is the rapidity of acquisition, with one-trial learning being commonly reported. In contrast, prior studies of aversion learning based on hypertonic saline have used multiple pairings, with conditioning effects only emerging after several trials (e.g. Lin et al., 2013). In Experiment 1, we examined the effects of pairing a palatable flavor with internal pain in rats using the analysis of the licking microstructure in a one-trial conditioning design. Rats were given access to a saccharin solution and immediately afterwards received a hypertonic NaCl injection, control rats received unpaired exposure to saccharin and hypertonic NaCl. Consumption and lick cluster size were recorded in training and across six extinction test sessions.

2.1 Method

2.1.1. Subjects

Thirty-two Lister Hooded rats, with a mean free-feeding weight of 362 g (range, 316-415 g) at the start of the experiment, were used. Rats were supplied by Harlan, UK and all procedures reported here were conducted in accordance with the Animals Scientific Procedures Act (1986) requirements for animal experimentation in the UK. Rats where housed in fours in standard (56 × 38 × 22 cm) plastic cages in a colony room under 12hr/12hr light/dark cycle (lights on at 07:30) and at an ambient temperature of 21º C. All experimental manipulations took place during the light phase and under an ad libitum food schedule. Before the start of the experiment, rats were moved to water restriction schedule with 60 minutes access to water in the home cage per day, given approximately one hour after the experimental sessions.

2.1.2. Fluids and apparatus

The CS was a 0.1% (w/w) sodium saccharin solution, and the US was sodium chloride (1.5 M NaCl) administered intraperitoneally (i.p.) at a volume of 10 ml/kg of body weight. Training and testing phases took place in a room contained 16 custom-made automated drinking chambers measuring 32 × 15 ×12 cm, with acrylic walls, steel mesh flooring and wire mesh lids. 50 ml drinking bottles with metal spouts could be inserted at one end of each box. A contact sensitive lickometer registered the licks made by rats to the nearest 0.01 s, and MED-PC software (Med Associates, Inc) controlled the equipment and recorded the data.

2.1.3. Procedure

Rats received two sessions of habituation to the experimental boxes before starting the training phase. In each session, they had access to a bottle containing water for 3 minutes. Rats were randomly assigned to two groups of 16: Group Control or Group Hypertonic (see Table 1). The training phase consisted of two 3-minute sessions (one per day), during which rats had access to either saccharin or distilled water. Group Hypertonic received hypertonic saline injection (1.5M, 10ml/kg) immediately after drinking saccharin (and no injection after drinking water). Group Control received a hypertonic saline injection (1.5M, 10ml/kg) after drinking water (and no injection after drinking saccharin). Half the rats in each group received saccharin on the first training day and water on the second, with the remainder receiving the solutions in the reverse order. Once the training was completed, rats received six test sessions (one per day) in which they had access to bottle containing the saccharin solution for 15 minutes.

2.1.4. Data analysis

Consumption was measured by weighting bottles before and after each experimental session. For the analysis of mean lick cluster size, a cluster was defined as a series of licks separated by pauses no more than 0.5 s interval, a criterion recommended by Davis (1989)

and used our previous studies of CTA and licking behavior (e.g., Dwyer, 2009; Dwyer, Burgess, & Honey, 2012; Dwyer, Gasalla, & López, 2013). Although alternative criteria have been used (e.g., 1 s, Spector, Klumpp, & Kaplan, 1998) parametric analysis have found little practical differences between them, given that most pauses greater than 0.5 s are also greater than 1 s (Davis & Smith, 1992). Data from saccharin consumption and lick cluster size in training were subject to independent *t* tests. Mixed analyses of variance (ANOVA) were used to analyze the test data with group as a between subject factor and a within-subject factor of extinction session. In addition, the assessment of lick cluster size requires at least some voluntary consumption. Because some rats displayed a total suppression of licking at the start of test, data from the first test session in which the rat reached a minimum criteria of 1 ml consumption was used for an additional analysis of lick cluster size (as an example, for some rats this was reached on first test session but for others reached it only on the fifth session). All tests reported here used a significance value of p = .05.

2.2. Results

Figure 1 shows the data from training and test sessions (consumption Panel A and lick cluster size Panel B). The groups did not significantly differ in either saccharin intake [$t(30) = 1.10$; $p = .279$] or lick cluster size to saccharin [$t(30) = .82$; $p = .420$] during training.

During test, Group Hypertonic showed lower saccharin intake than Group Control across all test sessions. ANOVA revealed main effects of test session, $F(5,150) = 47.03$, $p < .001$, group, $F(1,30) = 49.38$, $p < .001$, and a significant session by group interaction, $F(5,150) = 5.92$, $p < .001$. Simple main effect analyses revealed that Group Hypertonic displayed lower saccharin consumption than Group Control in all test sessions (lowest $F(1,30) > 15.51$, $p < .001$ for the last test session). Although mean lick cluster size was initially reduced in Group Hypertonic, by the end of testing there was no difference from Group Control. ANOVA revealed significant main effects of session, $F(5,150) = 23.7$, $p <$

.001, group, $F(1,30) = 19.61$, $p < .001$, and a significant session by group interaction, $F(5,150) = 6.20$, $p < .001$. An analysis of simple effects revealed that lick cluster size differed between groups during sessions 1 to 4, lowest $F(1,30) = 9.62$, $p = .004$ on session 4, but not during sessions 5 and 6 ($F(1,30) = 2.28$, $p = .142$ and $F(1,30) = 0.20$, $p = .656$ respectively).

In order to ascertain if the lick cluster analysis was affected by total suppression of licking behavior, a further analysis applied a minimum criterion of at least 1ml consumption. The data from the first session in which each individual rat reached this criterion was collated and analyzed for both lick cluster size and consumption. Rats in Group Control reached this criterion in a mean of 1.12 sessions (SEM 0.12), while Group Hypertonic reached it in a mean of 3.18 sessions (SEM = 0.40), $t(30) = 4.92$, $p < .001$. At this point, mean consumption in Group Control (8.00, SEM = 3.81) was higher than in Group Hypertonic (3.38, SEM = 1.74), $t(30) = 4.40$, $p < .001$. However, there was no longer a significant difference between the groups in terms of lick cluster size (mean Control = 26.41, SEM = 9.74; mean Hypertonic = 20.61, SEM = 13.11; $t(30) = 1.42$, $p = .166$).

In summary, pairing 0.1% saccharin solution with hypertonic NaCl reduced both consumption and lick cluster size – the consumption difference persisted across extinction testing while the lick cluster difference did not. This replicates the effects reported by Lin et al. (2013) and demonstrates that one-trial conditioning is possible with internal pain as the US, clearly demonstrating that rapid aversion learning is not restricted to nausea-producing treatments. Notably, there was no difference between groups in lick cluster size when the analysis was restricted to the first day in which rats consumed at least 1ml, while the consumption difference was maintained. This stands in contrast to studies of the extinction of LiCl-based aversions where at least some reduction in lick cluster size is still present after consumption has begun to recover (Dwyer, 2009; Dwyer et al., 2013).

3. Experiment 2

In Experiment 1, a single pairing of saccharin with the internal pain produced by hypertonic NaCl reduced both consumption and lick cluster size. However, the fact that the reduction in lick cluster size was dependent on strong suppression of consumption is a reminder that these lick analysis procedures rely on voluntary consumption and that mean lick cluster size might be difficult to assess reliably when there is very limited consumption. The taste reactivity method – through using intra-oral infusion of the cue solutions – ensures that rats are exposed to the CS without the need for voluntary consumption. Moreover, as noted in the introduction, the taste reactivity method has never been applied to the analysis of conditioning using hypertonic saline to produce internal pain.

Therefore, Experiment 2 used intraoral infusion and TR methods to compare the effects of pairing a flavor CS with either internal pain produced by hypertonic saline (1.5M, 4ml/kg) or nausea induced by LiCl (0.15M, 2ml/kg). These doses were chosen on the basis of pilot work suggesting that they have equivalent effects on solution consumption and do not produce complete 1-trial learning – thus affording an analysis of the development of conditioning. Rats received 4 training sessions in which intraoral infusions of a saccharin solution were followed by i.p. injections of hypertonic NaCl, isotonic NaCl or LiCl respectively (see Table 1). Orofacial responses to the solution were recorded in all training sessions and a single non-reinforced test, before being followed by a series of tests of voluntary consumption with the analysis of licking microstructure. In addition to the orofacial and somatic responses traditionally assessed using the TR test, we also analyzed immobility/freezing behavior as an index of conditioned fear (Bouton & Bolles, 1980; Dumigan, Lin, Good, & Honey, 2015).

3.1 Method

3.1.1. Subjects

Thirty male Wistar rats from the University of Oviedo vivarium (Spain) were used. They were approximately 90 days old and with a mean free-feeding weight of 331 g (range, 220-393 g) at the start of the experiment. Upon arrival, they were housed individually in standard (42 × 26 × 20 cm) 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 (210/63/UE) and Spanish regulation RD-53/2013 regarding the care and use of laboratory animals.

3.1.2. Fluids and apparatus

The fluids used as US were solutions of lithium chloride (0.15 M LiCl), isotonic saline (0.15 M NaCl solution), and hypertonic saline (1.5 M NaCl). LiCl was administered intraperitoneally (i.p.) at a volume of 2 ml/kg of body weight whereas hypertonic and isotonic NaCl were administered i.p. at a volume of 4ml/kg. The CS was a 0.1% (w/w) saccharin solution infused directly into the mouth of the subject through an oral cannula which had been implanted prior to the experiment.

Behavioral procedures took place in a conditioning chamber located in a dark room. The chamber was made of clear Plexiglas sides (26 × 23 × 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 rats 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. All the videos were analyzed by two independent raters.

3.1.3. Cannulation surgery

The rats were surgically implanted with an intraoral cannula using a very similar method to that described by Parker (1984, 1995a). Rats were anaesthetized with an i.p. injection of ketamine (50 mg/kg) combined with medetomidine clorhidrato (0.15 mg/kg). 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.

3.1.4. Procedure

Two rats lost their cannula during the experiment and were removed from it. The remaining rats were randomly assigned to three groups: Group Lithium (n=9); Group

Hypertonic (n=10); and Group Isotonic (n=9). All rats had recovered from the oral cannulation surgery within three days, and then 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).

Four days after surgery, rats were given a 1 minute session with water infusion in the conditioning chamber in order to habituate them to the apparatus and to the intraoral infusion method (infusion rate 1ml/min). The training phase consisted of four days for all rats (see Table 1). During each of the four training sessions, rats were placed in the conditioning chamber, and they were infused with the saccharin solution for two minutes (1ml/min). Immediately after the infusion was completed rats in Group Lithium were injected i.p. with LiCl (0.15 M, 2 ml/Kg); rats in Group Isotonic were injected with isotonic NaCl (0.15 M, 4 ml/Kg); and rats in Group Hypertonic with hypertonic NaCl (1.5 M, 4 ml/kg) before being returned to the home cage. The TR test occurred the next day, and was the same as conditioning with the exception that no injections were performed. On the next five days consumption tests were administered. In each of these sessions, the rats were given 15 minutes access to a drinking tube containing the saccharin in 8 boxes (similar to those described for Experiment 1) that were placed in the same room as the taste reactivity chamber. The amounts consumed were measured by weighting the bottles before and after consumption test and lick cluster size was analyzed as described previously.

Based on the procedure followed by Parker (1984, 1995a), 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). Forelimb flails (rapid horizontal movements of the forelimbs for remove fluid from the fur) and head shakes (rapid side-to-side head movements with the mouth open in order to remove the fluid out of the mouth) were also scored as aversive responses. 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 total 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 percentage of time of spent immobile over the infusion period (scored as suppression of all the movements in the rat with the exception of those required for respiration) was assessed to measure fear. The frequency of "passive-dripping" (each occasion on which a drop of fluid was allowed to leak out of the mouth to the floor without other orofacial actions) was also scored. Passive dripping and immobility were scored independently such that time spent dripping was not recorded as immobile. The inter-rater reliability for each behavior scored was highly significant (r´s > 0.81).

3.1.5. Data analysis

The TR behaviors during training and test were analyzed with 3 (group) × 5 (session) mixed ANOVAs. Appetitive, aversive, passive-dripping and immobility data were analyzed separately. A 3 (group) × 5 (session) mixed ANOVA was used to examine the consumption and lick cluster size data from the final bottle tests. While the main analysis was based on the sum of all the aversive responses scored here (that is, including the "mild" conditioned

responses such as head shaking and forelimb flails), we also performed supplementary analyses based on either gaping alone or on the "stronger" aversive responses (gaping, chin rubbing and paw treading combined) reflecting the analysis methods typically reported by Parker and colleagues.

3.2. Results

Figure 2 shows the data for the training and TR test session with intra oral infusion of the CS, as well as the bottle test sessions for voluntary consumption and cluster size. Figure 2A shows appetitive responses during training and test sessions. Both Group Hypertonic and Group Lithium displayed fewer appetitive responses to saccharin than to Group Isotonic. ANOVA revealed main effects of session, $F(4,100) = 14.04$, $p < .001$, group, $F(2,25) = 44.75$, $p < .001$, and a significant session by group interaction, $F(8,100) = 17.99$, $p < .001$. An exploration of this effect with pairwise comparisons revealed that groups did not differ in the number of appetitive responses displayed to saccharin on the first training session (largest $t(25) = 0.81$, $p = .426$ between Groups Isotonic and Lithium). But after one session Group Isotonic displayed more appetitive responses than Group Hypertonic (lowest $t(25) = 3.62$, $p = .001$ on session 2), and although Groups Isotonic and Lithium did not differ on the second training session ($t(25) = 2.04$, $p = .052$) they did from the third training session onwards (lowest $t(25) = 7.34$, $p < .001$ on session 3). Groups Lithium and Hypertonic showed equivalent levels of appetitive responses over all sessions (largest $t(25) = 1.53$, $p = .138$ on session 2).

Figure 2B suggests that Group Lithium displayed more aversive responses to the saccharin solution than Groups Hypertonic and Isotonic, which did not differ from each other. ANOVA revealed main effects of session, $F(4,100) = 7.89$, $p < .001$, group, $F(2,25) = 43.89$, $p < .001$, and significant session by group interaction, $F(8,100) = 5.64$, $p < .001$.

Pairwise comparisons revealed no differences between groups on the first session (largest $t(25) = 0.75$, $p = .461$ between Group Isotonic and Hypertonic). More importantly, Group Lithium displayed higher aversive responses on the remaining sessions than either Group Hypertonic (lowest $t(25) = 3.85$, $p = .001$ on session 2) or Group Isotonic (lowest $t(25) = 4.24$, $p < .001$ on session 2). Critically, Group Hypertonic and Isotonic showed equivalent aversive reactions all over the training and test sessions (largest $t(25) = 1.06$, $p = .298$ on session 3)[1].

Figure 2C shows immobility (indicative of fear). Groups did not differ on the first training session, and Group Hypertonic showed increased fear responses compared to Group Isotonic and Lithium over the remaining sessions. ANOVA revealed main effects of session, $F(4,100) = 13.57$, $p < .001$, group, $F(2,25) = 28.86$, $p < .001$, and a significant session by group interaction, $F(8,100) = 6.47$, $p < .001$. Pairwise comparisons revealed no differences between groups on the first session (largest $t(25) = 1.28$, $p = .213$ for the difference between Groups Hypertonic and Lithium). Group Hypertonic displayed higher immobility responses on the remaining sessions than either of Groups Isotonic (lowest $t(25) = 2.50$, $p = .019$ on session 2) and Lithium (lowest $t(25) = 3.19$, $p = .004$ on session 2). Groups Lithium and Isotonic did not themselves differ on immobility responses over training and test (largest $t(25) = 1.49$, $p = .149$ on session 4). Figure 2D shows passive dripping. As expected, groups did not differ on the first training session, while Group Hypertonic subsequently showed increased passive dripping compared to Groups Isotonic and Lithium, and the later showed more passive dripping responses than Group Isotonic. ANOVA revealed main effects of

[1] The same analysis carried out using either gaping alone, or gaping, chin rubbing, and paw treading combined as strongly aversive responses revealed the same results. That is, Lithium Group displayed more aversive responses to the saccharin solution than Group Isotonic and Hypertonic, which did not themselves differ.

session, $F(4,100) = 28.11$, $p < .001$, group, $F(2,25) = 54.81$, $p < .001$, and a significant session by group interaction, $F(8,100) = 10.82$, $p < .001$. Pairwise comparisons revealed no differences between groups on the first session (largest $t(25) = .79$, $p = .433$ for the difference between Groups Hypertonic and Isotonic). Group Hypertonic displayed more passive dripping on the remaining sessions than either of Groups Isotonic (lowest $t(25) = 3.98$, $p = .001$ on session 2) and Lithium (lowest $t(25) = 2.94$, $p = .007$ on session 2). Groups Lithium and Isotonic did not themselves differ on the second training session ($t(25) = 1.14$, $p = .320$), but they did from third session onwards (lowest $t(25) = 3.48$, $p = .002$ on session 3). Moreover, an analysis of the correlation between passive dripping and immobility responses in groups Hypertonic and Lithium revealed strong positive correlation, $r(17) = .93$, $p < .001$, [95% CI = .82, .97]. This may be consistent with passive dripping and immobility both reflecting conditioned fear, or with passive dripping being a secondary consequence of both fear and rejection of consumption.

Turning to the bottle consumption tests, Figure 2E shows saccharin intake. Group Isotonic initially had greater saccharin intake than both the Hypertonic and Lithium groups, with this difference decreasing over extinction testing. ANOVA revealed main effects of session, $F(4,100) = 59.47$, $p < .001$, group, $F(2,25) = 9.63$, $p = .001$, and a significant session by group interaction, $F(8,100) = 8.82$, $p < .001$. Pairwise comparisons revealed that Group Isotonic consumed more saccharin than Group Lithium on sessions 1-4 (lowest $t(15) = 2.52$, $p = .018$ for test 4), however, by the end of the extinction there were no differences between these two groups ($t(25) = 0.71$, $p = .482$ on test 5). Group Isotonic also consumed more than Group Hypertonic on sessions 1-2 (lowest $t(25) = 2.82$, $p = .009$ for test 2), but they had equivalent consumption on sessions 3-5 (highest $t(25) = 1.4$, $p = .173$ for test 3). Also, Group Hypertonic consumed more saccharin than Group Lithium on sessions 1-2

(lowest $t(25) = 2.46$, $p = .021$ for session 2) but they did not differ on sessions 3-5 (highest $t(25) = 1.29$, $p = .207$ for test 3).

Figure 2F shows that lick cluster size was higher for Group Isotonic than for Group Lithium, and this difference decreasing over extinction testing. ANOVA revealed main effects of session, $F(4,100) = 9.78$, $p < .001$, and a significant session by group interaction, $F(8,100) = 4.5$, $p < .001$, but there was no main effect of group, $F(2,25) = 1.49$, $p = .244$. Pairwise comparisons revealed that lick cluster size was larger for Group Isotonic than Group Lithium on session 1 ($t(25) = 4.06$, $p < .001$), but not on sessions 2-5 (largest $t(25) = 1.68$, $p = .106$ on test 2). There were no significant differences in lick cluster size between Groups Hypertonic and Isotonic on any test session (largest $t(25) = 1.79$, $p = .085$ on test 2).

In summary, pairing saccharin with either LiCl or hypertonic NaCl resulted in equivalent reductions in appetitive TR responses to saccharin infusion. Despite the equivalent effect on appetitive responses, Group Hypertonic did not display an increase in aversive TR responses and only group LiCl displayed more aversive responses compared to the isotonic control group. In addition, Group Hypertonic showed higher fear responses to saccharin infusion in terms of immobility than Group Lithium, and also displayed more "passive" dripping (which may be effectively an avoidance of the fluid, as rats do not swallow the solution, but let it dribble out of the mouth). Group Lithium showed intermediate levels of passive-dripping and immobility. While Groups Lithium and Hypertonic showed a decrease in voluntary consumption of the CS, this was larger in Group Lithium. Although lick cluster size was reduced for Group Lithium, there were no differences in lick cluster size between the Isotonic and Hypertonic Groups.

4. Experiment 3

Experiment 2 showed a dissociation between the effects of hypertonic NaCl induced pain and LiCl induced nausea. Despite equivalent effects on appetitive TR responses, LiCl increased aversive TR responses, while hypertonic NaCl did not; but hypertonic NaCl produced higher levels of immobility (fear) responses than did LiCl. However, both USs were administered at relatively low dose levels and voluntary consumption was more affected by LiCl than hypertonic NaCl. Therefore, Experiment 3 replicated the general methods of Experiment 2, but increased the dose levels of both hypertonic NaCl (1.5M at 10ml/kg) and LiCl (0.15M at 10ml/kg) to produce stronger overall conditioning effects.

4.1 Method

4.1.1. Subjects, fluids, and apparatus

Twenty-seven male Lister Hooded rats, weighing from 456 to 540 g (mean 490 g) at the start of the experiment were used. Rats were supplied by Harlan, UK and all procedures reported here where conducted in accordance with the Animals Scientific Procedures Act (1986) requirements for animal experimentation in the UK. Except otherwise stated, deprivation conditions, apparatus, and other procedural details were the same as in Experiment 2. Each subject was implanted with an oral cannula using the procedure described in Experiment 2. The flavor used during the experiment was a 1% (w/w) saline solution. The rats were injected with either 10 ml/kg of 0.15 M LiCl, 10 ml/kg of 1.5 M NaCl or isotonic saline (10 ml/kg). Subjects were randomly assigned to three groups (9 rats per group): Group Lithium, Group Hypertonic and Group Isotonic.

4.1.2. Procedure

The training phase was similar to that of Experiment 2 (see Table 1). In each of 4 daily sessions, the rats were placed in the conditioning chamber for 2 min while their orofacial responses were video-recorded during intraoral infusion of the CS solution.

Immediately afterwards, the subjects were injected i.p. with LiCl (Group Lithium), hypertonic NaCl (Group Hypertonic) or isotonic saline (Group Isotonic). The day following the final conditioning session consisted of a non-reinforced TR test. Rats were placed in the conditioning chamber and infused with the CS solution for two minutes while their orofacial responses were recorded. The next 4 sessions constituted voluntary consumption tests. Rats had daily access to a bottle containing the CS solution for 15 minutes. Consumption and the lick cluster size were analyzed as in Experiment 2.

4.2. Results

Figure 3 shows the data for the training sessions and TR test (appetitive, aversive, immobility, and passive-dripping) as well from the bottle tests (consumption and lick cluster size). Figure 3A shows appetitive TR responses: Groups Hypertonic and Lithium displayed an equivalent decrease in appetitive responses to the infusion of the CS across training and test compared to Group Isotonic. ANOVA revealed main effects of session, $F(4,96) = 35.40$, $p < .001$, group, $F(2,24) = 180.13$, $p < .001$, and a significant session by group interaction, $F(8,96) = 12.76$, $p < .001$. Pairwise comparison revealed no differences between groups on session 1 (largest $t(24) = 1.29$, $p = .629$ for the difference between Group Isotonic against Group Lithium). Group Isotonic displayed higher appetitive responses on the remaining training and test sessions than either of Groups Hypertonic (lowest $t(24) = 11.32$, $p < .001$, on session 2) and Lithium (lowest $t(24) = 9.21$, $p < .001$, on session 2). Groups Hypertonic and Lithium did not themselves differ on any session (largest $t(24) = 2.11$, $p = .136$ on session 2).

Figure 3B shows aversive TR responses: Group Lithium displayed more aversive responses to the CS across training and test than Groups Hypertonic and Isotonic (which did not differ). ANOVA revealed main effects of session, $F(4,96) = 4.04$, $p = .004$, group, $F(2,24) = 70.96$, $p < .001$, and a significant session by group interaction, $F(8,96) = 4.21$, $p <$

.001. Although there was a significant difference between Groups Lithium and Isotonic on session 1 ($t(24) = 2.69$, $p = .039$) there were no significant differences between Groups Hypertonic and Lithium ($t(24) = 1.73$, $p = .291$) or Groups Hypertonic and Group Isotonic ($t(24) = 0.96$, $p = 0.999$). More importantly, Group Lithium displayed more aversive TR responses on the remaining training and test sessions than either of Groups Hypertonic (lowest $t(24) = 4.14$, $p < .001$ for session 2) and Isotonic (lowest $t(24) = 4.26$, $p < .001$ for session 2). Moreover, Groups Hypertonic and Isotonic did not themselves differ on any session (largest $t(24) = 1.08$, $p = .864$ for the test session) [2].

Figure 3C shows the immobility data: While both of Groups Lithium and Hypertonic displayed more immobility (fear) than Group Isotonic across training and test, this effect was larger for Group Hypertonic. ANOVA revealed main effects of session, $F(4,96) = 44.60$, $p < .001$, group, $F(2,24) = 98.11$, $p < .001$, and a significant session by group interaction, $F(8,96) = 19.88$, $p < .001$. There were no differences between groups on session 1 (largest $t(24) = 1.14$, $p = .794$ for the difference between Groups Hypertonic and Lithium). Group Hypertonic were more immobile on the remaining sessions than either of Groups Lithium (lowest $t(24) = 3.78$, $p = .003$ for session 2) and Isotonic (lowest $t(24) = 5.37$, $p < .001$ for session 2). Groups Lithium and Isotonic did not differ on session 2 (largest $t(24) = 1.70$, $p = .306$) but they did differ on the remaining sessions (lowest $t(24) = 4.48$, $p = .001$ for session 3).

Figure 3D shows passive dripping: As with immobility Group Hypertonic showed increased passive dripping compared to Groups Isotonic and Lithium, and Group Lithium displayed more passive dripping responses than Group Isotonic. ANOVA revealed main

[2] Analyses based on gaping alone, or on the combination of strongly aversive responses of gaping, chin rubbing and paw treading revealed the same general pattern of effects: No differences between groups on the first training session, but subsequently Group Lithium showed more aversive responses to saccharin than either of Groups Hypertonic and Isotonic, which did not differ from each other.

effects of session, $F(4,96) = 60.17$, $p < .001$, group, $F(2,24) = 108.77$, $p < .001$, and a significant session by group interaction, $F(8,96) = 26.67$, $p < .001$. There were no differences between groups on session 1 (largest $t(24) = 1.47$, $p = .458$ for the difference between Group Lithium and Group Hypertonic). Group Hypertonic displayed more passive dripping on the remaining sessions than Group Isotonic (lowest $t(24) = 3.94$, $p = .002$ for session 2). Although Groups Hypertonic and Lithium did not differ on session 2 (largest $t(24) = 2.28$, $p = .095$), they did differ from session 3 onwards (lowest $t(24) = 7.34$, $p < .001$ for session 3). Groups Lithium and Isotonic did not differ on session 2 (largest $t(24) = 1.66$, $p = .327$ for the second session) but Group Lithium showed more passive dripping than Group Isotonic for the remaining sessions (lowest $t(24) = 4.46$, $p < .001$ for session 3). There was a strong positive correlation between passive dripping and immobility in Groups Hypertonic and Lithium, $r(16) = .71$, $p = .001$, [95% CI = .36, .88].

Figure 3E shows consumption and Figure 3F shows the mean lick cluster size from the bottle tests. Groups Hypertonic and Lithium consumed less of the CS compared to Group Isotonic, and these differences remained throughout testing. ANOVA revealed main effects of session, $F(3,72) = 44.04$, $p < .001$, group, $F(2,24) = 24.47$, $p < .001$, and a significant session and group interaction, $F(6,72) = 6.44$, $p < .001$. Group Isotonic consumed more saline solution over all sessions than either of Groups Hypertonic (lowest $t(24) = 2.89$, $p = .024$ on test 4) and Lithium (lowest $t(24) = 2.74$, $p = .034$ on test 4). Importantly, Groups Hypertonic and Lithium did not differ in consumption on any session (largest $t(24) = 1.51$, $p = .446$ for session 1). Lick cluster size was initially higher for Group Isotonic than for Groups Hypertonic and Lithium, however, no differences remained by the end of testing. ANOVA revealed main effects of session, $F(3,72) = 34.76$, $p < .001$, group, $F(2,24) = 10.65$, $p < .001$, and a significant session by group interaction, $F(6,72) = 3.33$, $p = .006$. Lick cluster size was larger for Group Isotonic than either of Groups Hypertonic (smallest $t(24) = 3.49$, p

= .006 on test 2) and Lithium (smallest $t(24) = 3.70$, $p = .003$ on test 2) for the first two sessions. There were no differences on the third and fourth test sessions between Group Isotonic and either Group Hypertonic (largest $t(24) = 2.52$, $p = .055$ on test 4) and Group Lithium (largest $t(24) = 2.49$, $p = .061$ on test 3). Unlike in Experiment 2, Groups Hypertonic and Lithium did not differ on any session (largest $t(24) = 1.39$, $p = .529$ on test 3).

In summary, pairing a CS solution with LiCl resulted in an increase in aversive TR responses to the CS, but pairing the same CS with hypertonic NaCl did not. In contrast, pairing the CS with hypertonic NaCl resulted in higher levels of fear (as indicated by immobility) than did pairing with LiCl. Notably, these divergent effects of LiCl and hypertonic NaCl occurred despite the two USs having equivalent effects on appetitive TR responses, voluntary consumption of the CS, and lick cluster size.

5. Discussion

The three experiments reported here examined the nature of the learning acquired when pain (produced by injection of hypertonic saline) or nausea (produced by injection of LiCl) are associated with a novel taste. Using the analysis of the lick cluster size, we found (Experiment 1) that a single pairing of saccharin solution with hypertonic NaCl injection resulted in both decreased consumption and lick cluster size, demonstrating for the first time that hypertonic NaCl can support learning as rapidly as LiCl. However, as soon as consumption had recovered to a minimal degree there was no remaining influence on lick cluster size. Experiments 2 and 3 represent the first examination of the effects of pairing a flavor CS with the US of internal pain produced by the injection of hypertonic NaCl using the TR test (they are also the first direct within-experiment comparisons of the effects of LiCl-induced nausea with internal pain produced by hypertonic NaCl in taste aversion conditioning). There was a clear dissociation between the effects of LiCl and hypertonic

NaCl in terms of conditioned aversive TR responses to the CS flavor: LiCl produced an increase while there was no difference between groups receiving hypertonic NaCl and controls receiving isotonic saline. There was also a clear, and opposite, dissociation in immobility (reflecting conditioned fear) to the CS: hypertonic NaCl produced a larger effect than LiCl. In addition, hypertonic NaCl produced larger effects on passive dripping than did LiCl. Critically, there were generally equivalent effects of both USs on appetitive taste TR responses to the CS. Although the effects of LiCl on voluntary consumption of the CS and on lick cluster size were larger than for hypertonic NaCl in Experiment 2, the effects were equivalent in Experiment 3 when higher US doses were examined.

The pattern of results across these experiments does not appear to be attributable to low levels of conditioning overall with internal pain produced by hypertonic NaCl injection: they occurred at both low (Experiment 2) and high (Experiment 3) doses of the relevant USs; there were effects in opposite directions for different measures – higher levels of aversive TR responses following LiCl compared to hypertonic NaCl, but higher levels of fear following hypertonic NaCl compared to LiCl; and they occurred in the context of equivalent effects on other measures (i.e. appetitive TR responses in Experiments 2 and 3, plus consumption and lick cluster size in Experiment 3). While these results are novel, they are not entirely unprecedented: cross experiment comparisons of the effects of pairing a CS flavor with LiCl, footshock, or lactose consumption (leading to lower gastrointestinal discomfort/pain) suggest that while all suppress voluntary consumption of the CS, only LiCl results in an increase in aversive TR responses (Pelchat et al., 1983; Simbayi, Boakes, & Burton, 1986). Therefore, there is clear evidence for selective conditioning: emetic treatments produce greater conditioned aversive hedonic responses than do pain-based non-emetic treatments, while pain produces larger effects on conditioned fear than do emetic treatments. In addition, there are many reports that pairing flavors with drugs of abuse results in marked decreases in

consumption and appetitive TR responses, but these treatments do not result in the same marked increase in aversive TR responses as seen with LiCl (Parker, 1995a; Parker, 2014).

Returning to the accounts of taste aversion learning outlined in the introduction, one feature of the analysis presented by Reilly and colleagues (Lin et al., 2014, 2016) is the suggestion that previously observed differences in the conditioned responses elicited by different USs might be quantitative, rather than qualitative. For example, internal pain or drugs of abuse might produce only a mild CTA (reflected in a reduction in appetitive TR responses and decreased lick cluster size, without an increase in aversive TR responses), while LiCl might produce a strong CTA (reflected in the fact that it is able increase aversive TR responses as well as decreasing appetitive TR responses). One possible implication of this analysis is that internal pain produced by hypertonic saline injection is generally lower in effectiveness/strength as a US in comparison to LiCl. But, as noted above, a general difference in US strength between LiCl and hypertonic NaCl cannot explain the current results. Obviously, we have direct evidence here only for the effects of internal pain as an example of a non-nausea negative US. So the degree to which the divergence from LiCl of other USs (in particular different drugs of abuse) might be explained in terms of a general difference in US strength remains to be determined.

However, an additional aspect of Reilly and colleagues analysis (Lin et al., 2014, 2016) is the suggestion that CTA can be conceived of as a general toxin avoidance mechanism and that the effects of pain or drugs of abuse might reflect “false positives” for this system. This idea was originally presented as a means of addressing the paradox that some drugs of abuse can support both CTA and conditioned preferences (especially for places/contexts). This false positive idea may also provide an explanation for the selective conditioning effects of different USs. If nausea-producing toxic compounds are the true target of the taste aversion system, then non-nausea events might (as false positives) only partially

recruit the mechanisms involved in conditioned taste aversion: in particular, they may not fully recruit whatever processes are involved in producing changes in hedonic reactions (while potentially fully recruiting processes involved in suppressing voluntary consumption). That is, differences in the ways that true and false positives for the toxin avoidance system are processed might explain the lower effects of hypertonic NaCl than LiCl on aversive hedonic responses despite the evidence of equivalent effects on other response measures. Obviously, this development of the false positive idea does not directly explain the fact that internal pain produced by hypertonic NaCl elicits larger effects than LiCl in terms of fear-related immobility, so some other process would need to be invoked to explain the difference in acquisition of fear responses between different USs (by analogy, one might suggest that nausea is a "false positive" for a danger avoidance mechanism).

Turning to the analysis by Parker (2003, 2014), the general idea that there are US-selective conditioning processes, where nausea-inducing events support "true" CTA (indicated by conditioned aversive TR responses) while non-nausea negative events support a fear-based process of taste avoidance learning (TAL – indicated by the suppression of consumption without aversive TR responses), is broadly consistent with the pattern of results observed here. It should be noted that the presence of fear-based TAL is (as the preceding sentence implies) normally inferred indirectly from the absence of aversive TR responses, rather than a direct assessment of fear responses. One exception to this "rule" is the observation that CS flavors previously paired with amphetamine potentiate acoustic startle responses, while CSs paired with LiCl attenuate them (Rana & Parker, 2007) – providing direct evidence that pairing a flavor with at least one non-nausea US provides it with fear-relevant properties. Interestingly, pre-treating with the anti-emetic ondansetron both reduces the display of aversive TR responses to a LiCl-paired CS (Limebeer & Parker, 2000) and allows LiCl-paired CSs to potentiate acoustic startle (Rana & Parker, 2007), despite having

no effect on voluntary consumption of the CS. This suggests that LiCl can (at least when its unconditioned or conditioned effects on nausea are suppressed) support the acquisition of fear-relevant behaviors. The current data add to this positive demonstration of conditioned fear with non-nausea USs through the increase in fear-related immobility following exposure to a CS paired with hypertonic NaCl (as well as showing lower levels of this response in LiCl paired cues).

While the current results are broadly consistent with Parker's suggestion that there are US-specific selective conditioning mechanisms, it should be noted that (as emphasised by Lin et al., 2014, 2016) both LiCl and hypertonic NaCl produced equivalent reductions in appetitive TR responses and lick cluster size. To the extent that reductions in these responses reflect a reduction in the hedonic value or palatability of the CS solution, then these results are inconsistent with the idea that TAL should not influence the hedonic evaluation of the CS. That is, evidence that hypertonic NaCl (or other non-emetic treatments) can induce conditioned negative hedonic responses might suggest that the difference between CTA and TAL is a difference of degree, rather than a difference of kind. Therefore, it is important to consider what might produce a change in appetitive TR responses and lick cluster size.

As outlined in the introduction, appetitive and aversive TR responses have been characterized 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) 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) (for an additional discussion of these issues see, Berridge, 2000; Berridge & Grill, 1983, 1984; Breslin, Grill, & Spector, 1992). The fact that hypertonic NaCl can reduce appetitive TR responses and lick cluster size to the same degree as LiCl, but only

LiCl increases aversive TR responses, has rather different implications in light of these two interpretations of the TR response measures: It may reflect either at lower level of reduction in overall hedonic response produced by hypertonic NaCl than LiCl, or it might indicate equivalent reductions in positive hedonic value for both USs with a dissociation in their effects on conditioned disgust. Moreover, response suppression secondary to immobility or avoidance of the solution might also contribute to the reduction in appetitive responses and lick cluster size. Thus, while there is clear evidence of US-specific selective conditioning effects, the question of whether this reflects qualitative differences of kind (e.g., Parker, 2003, 2014), or quantitative differences of degree (e.g., Lin et al., 2014, 2016), depends greatly on the way in which TR responses are interpreted.

While the current data does not directly discriminate between these different ideas, it does constrain the range of possible interpretations. A distributional analysis of appetitive and aversive TR responses across the development of LiCl-based CTA suggests that aversive TR responses such as gaping begin to emerge at low levels well before appetitive TR responses such as tongue protrusions or mouth movements stop being displayed entirely (Breslin et al., 1992). In Experiments 2 and 3, hypertonic NaCl reduced appetitive responses to floor levels without increasing aversive responses beyond those seen with the isotonic control. The absence of either aversive or appetitive TR responses by the end of training with hypertonic NaCl appears to be most consistent with either an external suppression of appetitive responses or a division between appetitive and aversive response classes. In order to further separate these possibilities, one direction for future research might be to use pharmacological means (e.g. anxiolytic drugs) to attenuate fear. Although there is evidence that common anxiolytics can produce a direct enhancement of taste palatability **(**e.g. Berridge & Treit, 1986; Parker, 1995b, for taste reactivity; Higgs & Cooper, 1997; Cooper & Higgs, 2005, for licking microstructure), larger effects of anxiolytic treatment on responses to pain over

nausea paired cues would suggest that there had been a suppression of responding through fear to pain-paired stimuli.

Putting the debate over the conceptual analysis of CTA to one side, our results also raise important considerations for the application of TR test and the analysis of the licking behavior. Firstly, the fact that in Experiment 1 conditioned reductions in lick cluster size were only seen when consumption was below 1ml is a reminder that very low levels of voluntary consumption necessarily limit the possible range of lick cluster sizes – making it potentially ambiguous as to whether low lick cluster size in such situations is a direct reflection of the hedonic value of the solution or the result of suppression of responding (or both). Similarly, the fact that appetitive TR responses are associated with an ingestive sequence of behaviors raises the possibility that they too might be susceptible to response suppression secondary to avoidance of the solution. Secondly, the tight correlation between immobility and passive dripping raises the possibility that dripping might not be a neutral response, but may reflect fear-based avoidance of the solution in some circumstances. Finally, the fact that conditioned reductions in lick cluster size extinguished before conditioned reduction in consumption implies that changes in consumption cannot be entirely due to changes in hedonic value. In this light, the current results are consistent with previous studies examining the extinction of changes in the palatability after taste aversion learning (Baird et al., 2005; Cantora, López, Aguado, & Rana, 2006; Davis, 1989; Dwyer, 2009; Dwyer et al., 2013) which report faster extinction for either lick cluster size or aversive TR responses than the avoidance of a previously conditioned flavor. The fact that extinction of lick cluster size changes is faster than the extinction of consumption reduction with both LiCl and hypertonic NaCl raises the possibility that, in extinction at least, there is a dissociation between the mechanisms underpinning changes in solution palatability and consumption.

In conclusion, while pairing a taste with either LiCl-induced nausea or internal pain produced by hypertonic NaCl results in the reduction in voluntary consumption of that taste (as well as reductions in appetitive TR responses and lick cluster size), these two negative events have clearly dissociable effects on other responses: only pairing with LiCl results in the production of conditioned aversive TR responses to the taste, while pairing with hypertonic NaCl results in the taste eliciting higher levels of immobility reflecting fear than does pairing the taste with LiCl. The different effects of nausea and internal pain cannot be attributed to general differences in the overall effectiveness of LiCl and hypertonic NaCl in supporting learning, but instead indicate US-specific selective conditioning effects. Whether this selective conditioning reflects differences of degree or of kind between the learning mechanisms engaged by emetic and non-emetic treatments in taste aversion remains to be fully determined. Regardless, associating a taste with internal pain may reduce the liking for that taste but, unlike pairing it with nausea, it does not make the taste actively disgusting, even when consumption is completely suppressed.

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Conflict of interest

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

Table 1

Table 1. Design of Experiments 1, 2, and 3

Experiment 1	**Train**		**Test**	
Hypertonic	Saccharin → 10ml/kg 1.5M NaCl	Water → Ø	6 × Saccharin	
Control	Saccharin → Ø	Water → 10ml/kg 1.5M NaCl		
Experiment 2	**Train**		**Test**	
			IO	**Bottle**
Lithium	4 × Saccharin (IO) → 2 ml/kg 0.15M LiCl		Saccharin	5 × Saccharin
Hypertonic	4 × Saccharin (IO) → 4 ml/kg 1.5M NaCl			
Isotonic	4 × Saccharin (IO) → 4 ml/kg 0.15M NaCl			
Experiment 3	**Train**		**Test**	
			IO	**Bottle**
Lithium	4 × Saline solution (IO) → 10 ml/kg 0.15M LiCl		Saline solution	4 × Saline solution
Hypertonic	4 × Saline solution (IO) → 10 ml/kg 1.5M NaCl			
Isotonic	4 × Saline solution (IO) → 10 ml/kg 0.15M NaCl			

Note. In Experiment 1, the order of the saccharin and water delivery was counterbalanced, such that half of the rats in each group received the saccharin first and the other half received the water first. IO: Intraoral infusions. Bottle represents voluntary consumption with both intake and licking microstructure recorded. LiCl and NaCl injections were administered intraperitoneally. CS: saccharin (0.1%) and saline solution (NaCl 1%).

Figure Legends

Figure 1. Experiment 1 data over training and test sessions for Groups Hypertonic and Control. Mean saccharin intake (Panel A) and mean lick cluster size (Panel B). Error bars represent the standard error of mean (*SEM*).

Figure 2. Experiment 2 data for Groups Lithium, Hypertonic, and Isotonic. Panels A-D reflect the data from the intraoral conditioning and test sessions: Panel A, mean duration of appetitive taste reactivity (TR) responses; Panel B, mean number of aversive TR responses; Panel C, mean time spent immobile as a percentage of the total time tested; Panel D, mean number of passive dripping events. Panels E and F reflect the data from voluntary consumption tests: Panel E, mean saccharin intake; Panel F, mean lick cluster size. Error bars represent the standard error of mean (*SEM*).

Figure 3. Experiment 3 data for Groups Lithium, Hypertonic, and Isotonic. Panels A-D reflect the data from the intraoral conditioning and test sessions: Panel A, mean duration of appetitive taste reactivity (TR) responses; Panel B, mean number of aversive TR responses; Panel C, mean time spent immobile as a percentage of the total time tested; Panel D, mean number of passive dripping events. Panels E and F reflect the data from voluntary consumption tests: Panel E, mean saline intake; Panel F, mean lick cluster size. Error bars represent the standard error of mean (*SEM*).

Figure 1

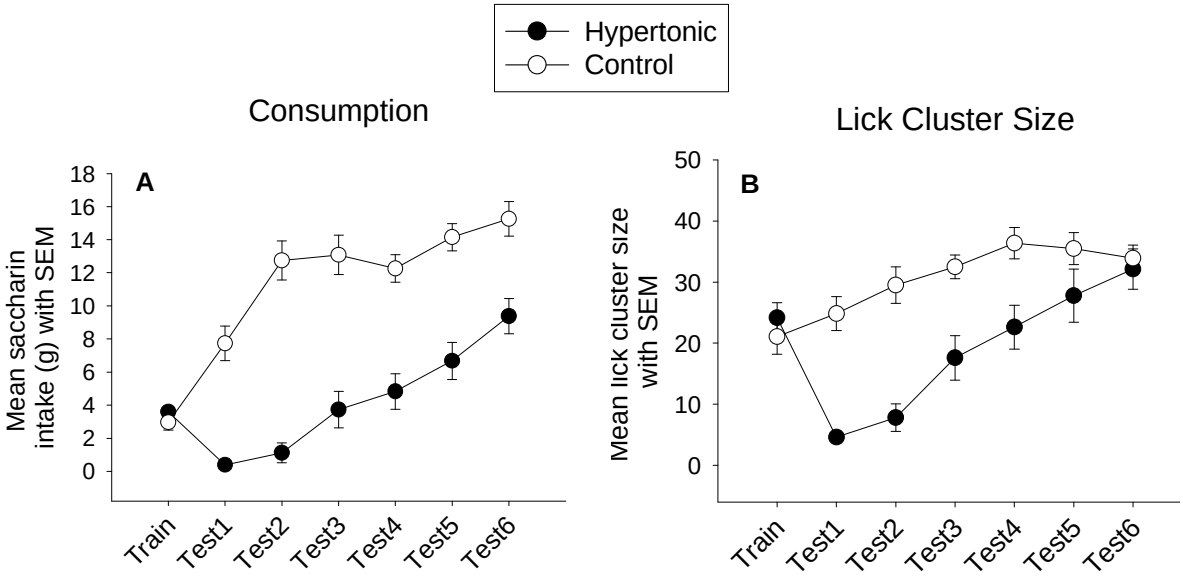

Hypertonic
Control
Consumption
A
Mean saccharin intake (g) with SEM
0
2
4
6
8
10
12
14
16
18
Train
Test1
Test2
Test3
Test4
Test5
Test6
Lick Cluster Size
B
Mean lick cluster size with SEM
0
10
20
30
40
50
Train
Test1
Test2
Test3
Test4
Test5
Test6

Figure 2

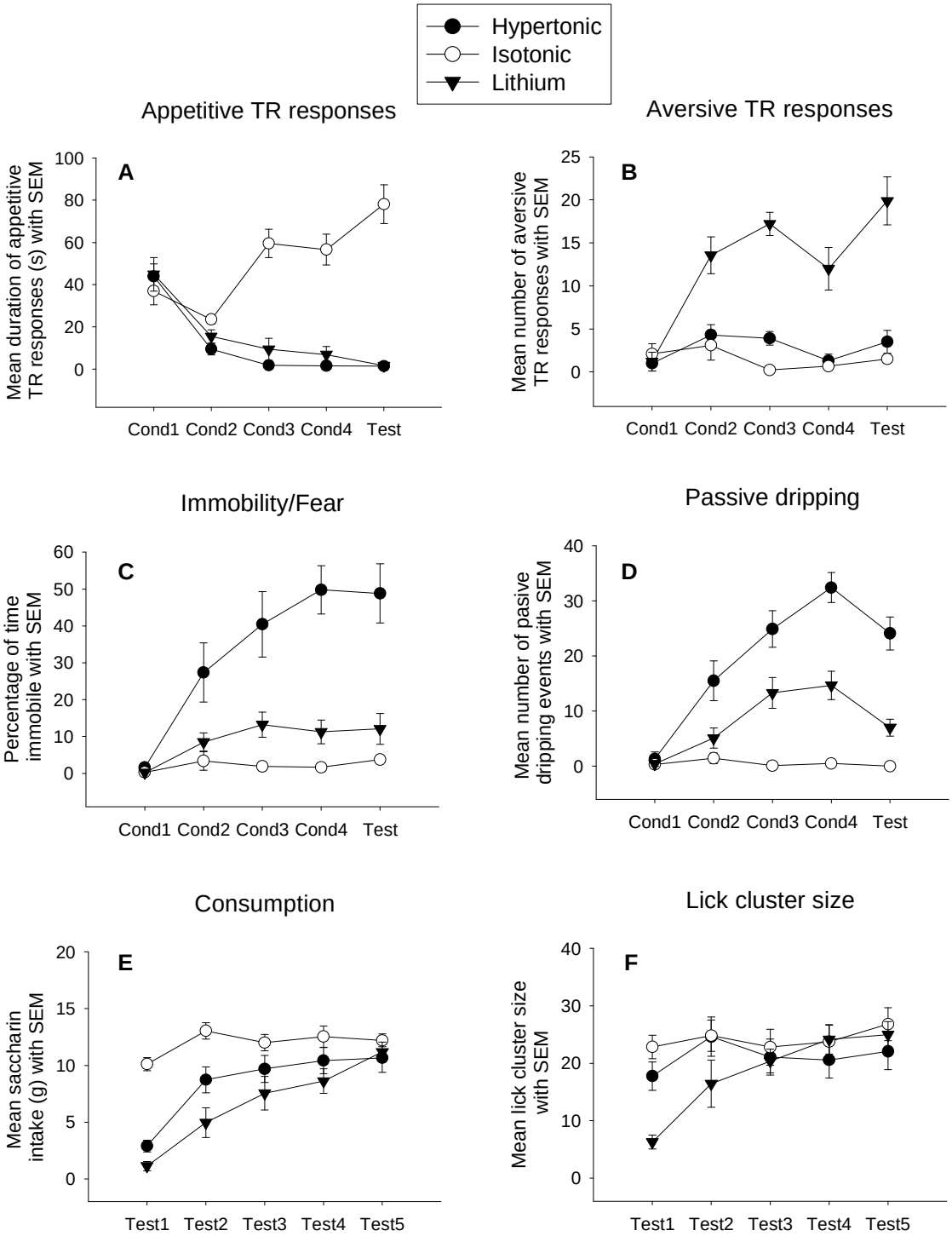
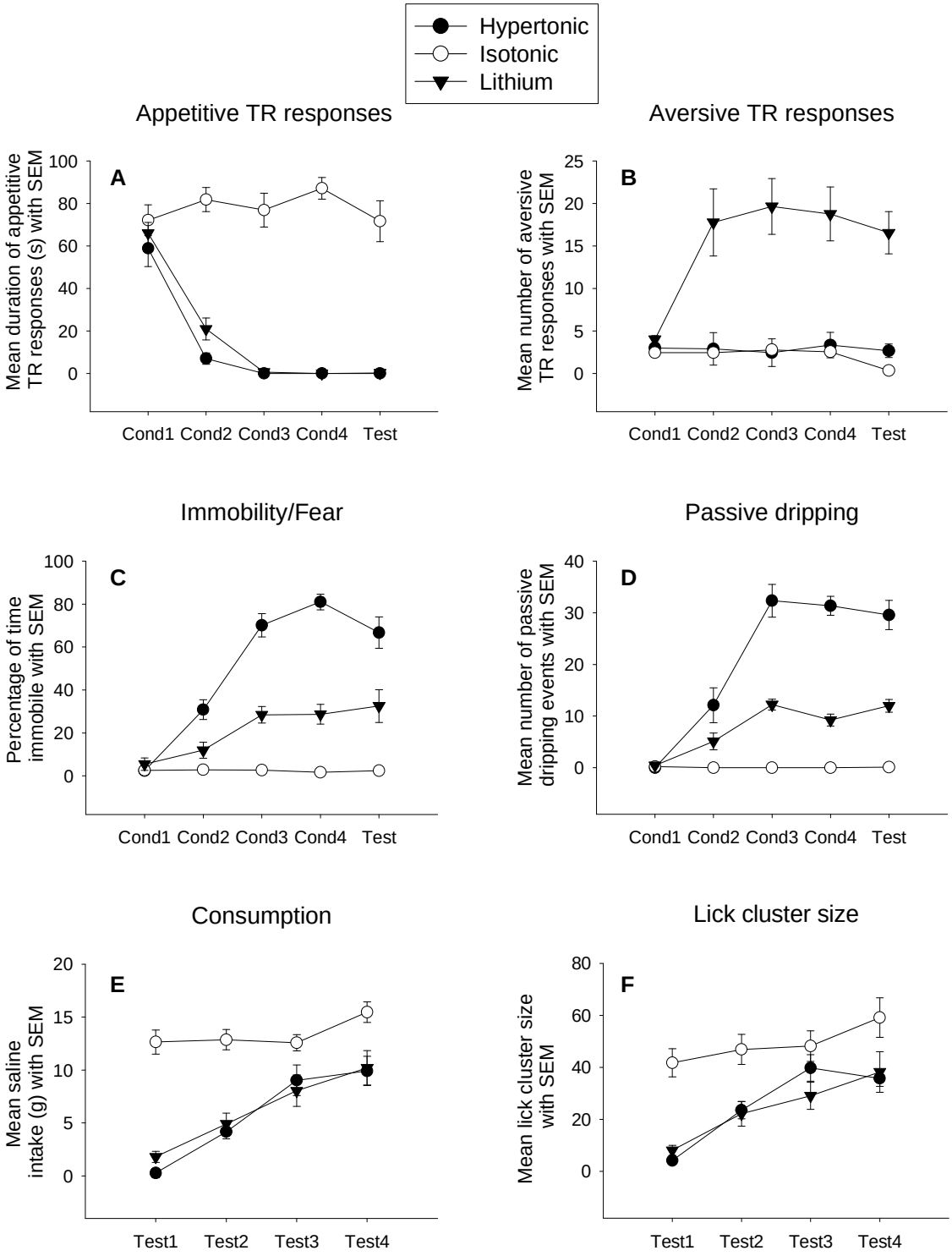

Hypertonic
Isotonic
Lithium
Appetitive TR responses
Aversive TR responses
A
B
Mean duration of appetitive TR responses (s) with SEM
Mean number of aversive TR responses with SEM
Cond1 Cond2 Cond3 Cond4 Test
Immobility/Fear
Passive dripping
C
D
Percentage of time immobile with SEM
Mean number of pasive dripping events with SEM
Consumption
Lick cluster size
E
F
Mean saccharin intake (g) with SEM
Mean lick cluster size with SEM
Test1 Test2 Test3 Test4 Test5

Figure 3


Hypertonic
Isotonic
Lithium
Appetitive TR responses
Aversive TR responses
A
B
Mean duration of appetitive TR responses (s) with SEM
Mean number of aversive TR responses with SEM
Cond1 Cond2 Cond3 Cond4 Test
Immobility/Fear
Passive dripping
C
D
Percentage of time immobile with SEM
Mean number of passive dripping events with SEM
Consumption
Lick cluster size
E
F
Mean saline intake (g) with SEM
Mean lick cluster size with SEM
Test1 Test2 Test3 Test4

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