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Odor-taste pairings lead to the acquisition of negative hedonic qualities by the odor in aversion learning

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ABSTRACT

Three experiments examined the affective responses conditioned to an odorous stimulus in the taste-mediated odor aversion learning paradigm. Experiment 1 analyzed the microstructure of licking behavior during voluntary consumption. Before conditioning, water-deprived rats had access to a bottle containing either a tasteless odor (0.01% amyl acetate) diluted in water or mixed with 0.05% saccharin. Next, the rats were injected with either LiCl or saline immediately after drinking saccharin. At test, they received the odor and taste solutions on separate days. Lick cluster size was used as a direct measure of the hedonic response to the odor cue. Rats receiving odor-taste pairings prior to the saccharin devaluation showed both lower consumption and lick cluster size, reflecting a reduced hedonic evaluation of the odor. Experiments 2a and 2b used the orofacial reactivity method. After pretraining in the drinking boxes with the odor alone or mixed with saccharin, the rats were intraorally infused with saccharin before injection with LiCl or saline. At test, they were infused in separate sessions with the odor and taste and their orofacial reactions video recorded. There were increased aversive orofacial responses to the odor in rats that had prior odor-taste experience, a result indicating a negative hedonic evaluation of the odor. These results provide evidence of conditioned changes in affective value of odor cues through taste-mediated learning and are consistent with the idea that odor-taste pairings lead to the acquisition of taste qualities by the odor.

1. Introduction

Over the past few years, a great number of publications have dealt with behavioral, neural, and affective aspects of flavor learning [1–3], and particularly flavor aversion learning [4]. Flavor perception[1] is a complex process that requires the integration of different sensory properties of foods - in particular olfactory and gustatory qualities, primary through the action of associative learning [5,6]. The olfactory aspects of flavors also play an essential role in the acceptance and consumption of foods. However, despite its importance in flavor learning, little is known regarding the contribution of odor hedonics to flavor learning and particularly to its influence on the development of conditioned taste aversions.

In rodents, the hedonic evaluation of odors, as with flavors, is most commonly inferred from behavioral tasks such as olfactory discrimination and odor preference learning [7,8], usually by assessing the amount of solution ingested. However, ingestive behavior may be influenced by motivational or physiological factors. In contrast to non-specific intake-only measures, conditioned changes in affective value of odors and flavors can be measured directly and selectively using behavioral methods such as the orofacial reactivity test[2] [9]. In this test, rats, and other species of rodents (mice, shrews) are implanted with intraoral cannulas and the orofacial and somatic responses accompanying an intraoral infusion of the flavor are recorded. This supports a direct examination of the hedonic evaluation or palatability of the infused solution. The orofacial responses elicited by the fluid can be classified as appetitive reactions such as tongue protrusions and paw licks (elicited, for example by pleasant, sweet tastes) or aversive reactions such as

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[1] Although 'taste' is often used as a synonym for 'flavor' because flavors are always experienced in the mouth, flavor is defined here as the result of the joint stimulation of the senses of smell and taste.

[2] This method was originally described as the taste reactivity test because it is most commonly applied to taste stimuli. We have chosen to emphasize the nature of the elicited responses – orofacial reactions – because we are considering its application to odor cues.

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gaping, chin rubbing, and paw treading (elicited, for example by unpleasant sour or bitter tastes). These patterns of orofacial reactions are universal across many species including human infant, primates, and rodents [10]. For the purposes of this study, it is important to point out that many species (including humans) readily learn to avoid fluids paired with toxins that have previously caused them gastrointestinal malaise, a phenomenon termed conditioned taste aversion (CTA) [4]. In rats, for example, pairing a palatable taste with nausea (e.g., produced by the administration of emetic drugs as lithium chloride) not only results in reduced consumption of that taste but also produces a reduction in its hedonic value or palatability [11]. When intraorally infused with a taste previously paired with LiCl, rats display aversive orofacial reactions reflecting a shift in the hedonic value of the taste from positive to negative [12,13].

The hedonic quality of flavors can also be assessed by analyzing the microstructure of licking behavior during voluntary consumption [14, 15]. The ingestive behavior of rodents consuming fluids consists of sustained runs of licks separated by pauses of varying length (clusters), and the mean number of licks in a cluster (lick cluster size) is directly related to the nature and concentration of the solution ingested. Lick cluster size shows a positive monotonic relationship to the concentration of palatable sweet solutions, while lick cluster size decreases monotonically with increasing concentration of unpalatable quinine solutions. In the context of conditioned taste aversions, pairing an otherwise palatable taste with nausea results in a reduction of lick cluster size similar to that produced by exposure to quinine. In addition, the pattern of licking behavior is sensitive to changes in the hedonic value of taste stimuli produced by physiological and pharmacological treatments known modulate palatability in humans. For example, sodium depletion elevates the palatability of sodium chloride, and the administration of benzodiazepines drugs, which increase appetitive responses in the taste reactivity test, enhance lick cluster size [16,17].

There is also some evidence that odors have the potential to be associated with nausea producing a change from neutral to aversive in the hedonic value of the odorant [18–20]. In the conditioned odor aversion (COA) paradigm, rats are given exposures to an odor stimulus (usually an ingested tasteless aqueous solution of odorant) followed by toxicosis, resulting in subsequent odor avoidance through the development of an association between olfactory information with the emotional aspects (i.e., negative hedonics) elicited by toxicosis.[3] In addition, it has been suggested that odors can acquire a specific taste quality after odor-taste experience. Gautam and Verhagen [5], for example, found that odor-taste pairings before conditioning of only the odor cue results in avoidance of both the odor and the sucrose, a result that was interpreted as indicating that the odor was perceived as sweet (i.e., sucrose-like) during conditioning. However, as noted above, in these studies the odors hedonic quality was inferred from a reduction in consumption but not by examining directly the affective responses elicited by odor solution. Therefore, the focus of the present study is to understand how odor aversion learning changes the hedonic valence of the odor and to what extent these changes depend on how the odor was first experienced (either as an odorant alone or mixed with a taste).

Here, we used a variant of the odor aversion (more specifically taste-mediated odor aversion) paradigm which has been previously used to study the hedonic qualities acquired by odors during conditioning [21]. Through associative learning, auditory, visual and olfactory cues paired with flavors that have been previously associated with toxicosis can acquire aversive properties, a phenomenon named mediated learning [22,23]. In our protocol, rats first received pairings of a tasteless odorant (amyl acetate) with a novel taste (saccharin) to endow the odor with the ability to activate an internal representation of the taste. The presentation of the taste alone was then paired with LiCl-induced nausea. After this conditioning, an odor test was given in the absence of the taste to assess mediated learning of an odor aversion. Direct aversion to the taste was also assessed. We were particularly interested in evaluating whether after initial odor-saccharin pairings, devaluation of the saccharin with LiCl leads to a reduction in the hedonic value of the odor. Different mechanisms could contribute to odor-taste interactions in aversion learning, such as mediated learning involving associatively activated representations, sensory preconditioning as a chain of associations, and generalization to the odor of the conditioned properties, including hedonic responses, acquired by the saccharin during conditioning. This last idea implies that the odor could acquire the taste properties of saccharin during odor-saccharin pairings. In the current experiments, we examine this issue by using licking behavior analysis (Experiment1) and orofacial reactivity methods (Experiments 2a and 2b).

2. Materials and methods

The general methods are presented first followed by specific details of the behavioral procedures of each experiment.

2.1. Subjects

Male Wistar rats (University of Oviedo vivarium, Spain), approximately 12 weeks old and weighing 303–398 g at the beginning of the experiment, were used. Upon arrival, the rats were housed individually in opaque plastic cages in a room maintained at 21 °C with a 12-hour light-dark cycle with the light on at 8:00 am. All experimental manipulations were performed during the light portion of the cycle. Before of the start the experiments, the rats were moved to a water-deprivation schedule with 60-min access to water in the home cage per day, given approximately 1 h after the experimental sessions. Food was always available in the home cages. All procedures reported here were conducted in accordance with Spanish (RD 53/2013) and European (2019/63/UE) legislation for animal experimentation.

2.2. Fluids

The conditioned stimuli (CSs) used were an odorant (0.01% amyl acetate, natural, purity ≥ 97%, Sigma-Aldrich) and a taste (0.05% saccharin, purity ≥ 99%, Merck), both dissolved in distilled water. The odor concentration was chosen because amyl acetate is known to be tasteless up to 0.1% to male Wistar rats [17]. The unconditioned stimulus (US) solution was lithium chloride (0.15 M LiCl, VWR Prolabo) administered intraperitoneally (i.p.) at a volume of 10 ml/ kg of body weight. Control rats were injected with isotonic saline (0.15 M at 10 ml/kg). In Experiment 1 the CS solutions were orally ingested by the rats in drinking tubes. In Experiments 2a and 2b, the CSs were intraoral infused through a cannula implanted into the mouth of the rats (see cannulation surgery section).

2.3. Apparatus

In Experiment 1 the behavioral procedures took place in a room containing 12 custom-made drinking boxes measuring 42 × 25 × 20 cm, with acrylic walls and floor, 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.

In Experiments 2a and 2b, the apparatus (drinking boxes) used for pretraining was as described for Experiment 1. Conditioning and testing sessions took place in a conditioning chamber (taste reactivity apparatus) 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

[3] Odorants can reach olfactory receptors by two routes: orthonasally, when volatiles enter the nasal cavity during inhalation, and retronasally, when food volatiles released in the mouth pass into the nasal cavity during consumption. 'Odor perception', in this paper, always refers to retronasal olfaction.

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 45° angle facilitated viewing of the ventral surface of the rat during the intraoral infusion. CS 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 10 X, model HDR-CX105) 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, which were blind to the experimental treatments, and the interrater reliability calculated for each response.

2.4. Cannulation surgery

To implant intraoral cannulas [24–26],[4] the rats were anesthetized with an i.p. injection of ketamine (50 mg/kg) combined with metedomidine hydrochloride (0.15 mg/kg). A thin-walled 15-gauge stainless steel needle was inserted at the back of the neck and guided underneath the skin and brought out behind the first molar inside mouth. A length of intramedic polyethylene tubing (PE-90, Becton Dickinson) with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was then run through the needle after which it was removed. The tubing was held secure in the oral cavity by an O-ring, which was sealed behind the tubing prior to cannulation surgery. After surgery, the cannulas were flushed daily with a solution of chlorhexidine (0.5%) to prevent infection. This flushing was done for three days before starting the experimental sessions. During monitoring, the rats were administered ketofren (1.5 mg/kg, s.c.), an anti-inflammatory drug, and the antibiotic enrofloxacin (0.3 mg/kg, s.c.). 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.

2.5. Orofacial response scoring

Based on the procedure followed by Parker [27,28], and as previously used in our studies [24–26], the aversive behaviors scored included the frequency of the responses of gaping (rapid, large-amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward) and paw treading (forward and backward movement of the forepaws in synchronous alternation). These scores were summed to provide a total aversive response score. The appetitive responses scored were tongue protrusions (extension of the tongue out the mouth), mouth movements (movement of the lower mandible without opening the mouth), and paw licks (midline extension of the tongue directed to the forepaws). The number of seconds that the rats displayed the responses was used as the appetitive response score. It should be noted that appetitive and aversive orofacial 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 [10].

2.6. Behavioral procedures

2.6.1. Experiment 1: licking behavior analysis

Experiment 1 evaluated the hedonic qualities acquired by an odor in the taste-mediated odor aversion paradigm by examining the microstructure of licking behavior. In a previous (unpublished) study from our laboratory water-deprived rats had access to a bottle containing an odorous solution (amyl acetate) before being injected with LiCl or saline. All rats subsequently received extinction sessions in which they were given the odor solution without aversive consequences. During conditioning and test sessions odor consumption and lick cluster size (as an index of hedonic evaluation of fluids), were recorded. Reductions in both consumption and lick cluster size were observed in rats injected with LiCl, suggesting that the odor had acquired negative hedonic properties after aversive conditioning.[5] In that previous study, the odor cue was directly paired with lithium-induced nausea. Here, we evaluated the hedonic qualities acquired by the odor using the taste-mediated odor aversion paradigm, i.e., after conditioning an aversion to a taste previously paired with the odor. It was expected that rats receiving odor-taste pairings before taste conditioning would reduce both odor consumption and lick cluster size compared to rats given prior experience with the odor alone.

The experimental design is summarized in Table 1. Rats ($N = 32$) were randomly assigned to one of four groups ($n = 8$) based in their weight, to balance weights across groups: Group OT-E (odor + taste, experimental), Group O-E (odor, experimental), Group OT-C (odor + taste, control), and Group O-C (odor, control). Before the start of the experiment the rats were given three sessions of habituation to the drinking boxes in which they had access to a bottle containing water for 10 min. The pretraining phase consisted of four 10-min sessions (one per day) during which rats had access to either the odorant alone (groups O-E and O-C) or odor mixed with saccharin (groups OT-E and OT-C). On the conditioning trial, the rats were given saccharin in the drinking boxes for 10 min before being injected with either lithium (groups OT-E and O-E) or saline (groups OT-C and O-C). After the conditioning session, the rats received a recovery day in which they were given water for 23 h in their home cages. On the next two test sessions (one per day), mediated and direct aversions were assessed. On Test 1 (mediated), the rats had access to a bottle containing the odorant, i.e., the stimulus previously associated with the lithium-paired taste; on Test 2 (direct),

Table 1
General design of experiments.

Experiment 1. *Licking behavior analysis;* Experiment 2a. *Orofacial reactivity test*				
Group	*Pretraining*	*Conditioning*	*Test 1 (mediated)*	*Test 2 (direct)*
OT-E	Amyl + Sac	Sac → Li	Amyl	Sac
O-E	Amyl	Sac → Li	Amyl	Sac
OT-C	Amyl + Sac	Sac → Sal	Amyl	Sac
O-C	Amyl	Sac → Sal	Amyl	Sac
Experiment 2b. *Orofacial reactivity test*				
Group	***Pretraining***	***Conditioning***	***Test 1 (mediated)***	***Test 2 (direct)***
OT-E	Amyl + Sac / Water	Sac → Li	Amyl	Sac
O/T-E	Amyl / Sac	Sac → Li	Amyl	Sac
O/T-C	Amyl / Sac	Sac → Sal	Amyl	Sac

Keys. OT: rats receiving the odor + taste compound during pretraining; O/T: rats receiving odor and saccharin in alternate days during pretraining; O: rats receiving odor alone during pretraining; E: experimental, rats injected with LiCl; C: control, rats injected with saline; Li and Sal indicate injections of LiCl or saline; Amyl refers to the odorant amyl acetate; Sac refers to saccharin. In Experiment 1, pretraining, conditioning, and tests 1–2 were conducted in the drinking boxes; In Experiments 2a and 2b, pretraining was conducted in the drinking boxes, and conditioning and testing in the taste reactivity apparatus.

[4] For a detailed description of the taste reactivity method, see [26] M. López, D.M. Dwyer, P. Gasalla, C. Jove, A. Begega. Characterizing hedonic responses to flavors paired with internal pain and nausea through the taste reactivity test in rats. Bio-protocol 12(18) (2022) e4515. https://doi.org/10.21769/BioProtoc.4515.

[5] This result was observed using the retronasal mode of olfaction but not when the odor was perceived by orthonasal stimulation. For this reason, here the odorant was delivered by the retronasal route.

they were given the lithium-paired taste for 10 min. Consumption and lick cluster size were recorded across the experimental sessions. During testing, three rats displayed a total suppression of licking and were removed from the experiment because the assessment of lick cluster size requires at least some voluntary consumption. Thus, the final group sizes were Group OT-E ($n = 8$), Group O-E ($n = 7$), Group OT-C ($n = 7$), and Group O-C ($n = 7$).

2.6.2. Experiments 2a and 2b: orofacial reactivity test

Experiments 2a and 2b used the orofacial reactivity method to examine affective responses elicited by the odor. Experiment 2a replicated the design of Experiment 1 (see Table 1) with the only difference that the rats received intraoral infusions of the CS fluids during conditioning and testing. Experiment 2b evaluated whether the conditioned properties (including affective responses), acquired by the saccharin after its pairing with LiCl would generalize to the odor cue because the saccharin has both taste and olfactory components which are presumably available for conditioning.

In Experiment 2a, the rats ($N = 40$) were randomly assigned to four groups ($n = 10$): Group OT-E (odor + taste, experimental), Group O-E (odor, experimental), Group OT-C (odor + taste, control), and Group O-C (odor, control). The pretraining phase was similar to that of Experiment 1 (including using the same fluid restriction schedule). On each of four sessions the rats had access voluntary bottle access to either the odor alone (groups O-E and O-C) or the odor-saccharin compound (groups) OT-E and OT-C) for 10 min. The rats were then supplied with water and food in their home cages prior to cannula implantation. Four days after the surgery, the rats were returned to the water deprivation-schedule, comprising 1 h access to water each day. They were then given a 1 min session with water infusion in the taste reactivity chamber to habituate to the apparatus and to the intraoral infusion method (infusion rate 1ml/min). The next two days constituted the conditioning phase. The rats received two conditioning trials separated by a recovery day during which they were given water for 23 h in their home cages. On each of the conditioning trials, the animals were placed in the taste reactivity chamber and intra-orally infused with the saccharin solution (0.05%) for 2 min at a rate of 1 ml/min while their orofacial responses were recorded. Immediately following the fluid infusion, the rats in groups OT-E and O-E were injected (i.p.) with LiCl whereas those in groups OT-C and O-C received an injection of isotonic saline. On the next two days the test sessions were conducted. During these sessions, each rat was placed in the taste reactivity chamber with their cannula attached to the infusion pump. On Test 1 (mediated aversion) the animals were intraorally infused with the odor solution (amyl acetate) for 2 min at a rate of 1 ml/min. On Test 2 (direct aversion), the rats were infused with the saccharin for another 2 min (rate 1 ml/min). During the fluid infusions the rats´ orofacial responses were recorded.

In Experiment 2b, the rats ($N = 30$) were assigned to three groups ($n = 10$): Group OT-E (odor + taste, experimental), Group O/T-E (odor/taste, experimental), Group O/T-C (odor/taste, control). After habituation to the drinking boxes, rats were given exposures to the odor and the saccharin solutions for 10 min over 10 days. On odd days, rats in Group OT-E were given the odor-saccharin mixture, while rats in groups O/T-E and O/T-C received exposures to the odor dissolved in water. On even days, rats in the paired group were given water, and those in the unpaired groups received saccharin exposures. Conditioning and testing sessions were as described for Experiment 2a. For the conditioning phase, the rats were infused with saccharin for 2 min and injected with either lithium (groups OT-E and O/T-E) or saline (group O/T-C). On testing, each rat was infused with the odor and the saccharin solutions for 2 min on successive days. Two rats lost their cannula during the experiment and were removed from the sample, and as a result, the final group sizes were: Group OT-E ($n = 10$), Group O/T-E ($n = 9$), and Group O/T-C ($n = 9$).

2.7. Statistical analysis

In Experiment 1, fluid consumption was measured by weighing 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 0.5s duration, a criterion used in our previous studies examining taste aversion learning by licking analysis [24,29]. Data from consumption and lick cluster size during pretraining were analyzed with 2 (fluid: odor vs odor-taste) x 2 (conditioning: saccharin paired with LiCl *vs* saline) x 4 (trial) mixed ANOVAs. Data from the conditioning and test sessions were analyzed by separate 2 (fluid) x 2 (conditioning) ANOVAs. Where informative, follow up analyses were performed as pairwise comparisons reflecting the 2 by 2 between-subject aspect of the design (here and in Experiment 2a which used the same general design).

In Experiments 2a, data from consumption and cluster size during pretraining sessions were analyzed with 2 (fluid: odor vs odor-taste) x 2 (conditioning: saccharin paired with LiCl vs saline) x 4 (trial) mixed ANOVAs. The orofacial reactivity responses displayed by the rats during conditioning were analyzed with 2 (fluid) x 2 (conditioning) x 2 (trial) mixed ANOVAs (here, and with all orofacial reactivity analyses, there were separate analyses for the appetitive and aversive reactions). Data from Test 1 and Test 2 were analyzed by separate 2 (fluid) x 2 (conditioning) ANOVAs. In Experiment 2b, data from pre-training during odd and even days were analyzed by separate 3 (group) x 5 (trial) ANOVAs. Data for conditioning sessions were analyzed by a 3 (group) x 2 (trial) mixed ANOVA. Orofacial responses elicited during Test 1 (odor) and Test 2 (saccharin) were analyzed by separate one-way ANOVAs, with group as between-group factor. Where informative, follow-up analyses were performed as pairwise comparisons among the three between-subject groups. The interrater reliability ($rs > 0.85$) for each behavior scored was highly significant. The different scales on which the aversive and the appetitive responses are scored requires that each is analyzed separately. All tests reported here used a criterion for significance of $p = .05$.

3. Results

3.1. Experiment 1: licking behavior analysis

The mixed ANOVA performed with the consumption data during pretraining revealed significant main effects of trial, $F(3,75) = 66.88$, $p < .001$, and fluid, $F(1,25) = 25.22$, $p < .001$, but not a significant effect of conditioning, $F(1,25) = 2.21$, $p = .149$. The trial x fluid interaction was significant $F(3,75) = 29.50$, $p < .001$, but there was neither a significant trial x conditioning interaction, nor a significant interaction of the three factors (largest $F(3,75) = 1.31$, $p = .277$). An exploration of the trial x fluid interaction with pairwise comparisons revealed that group OT-E displayed higher consumption than group O-E on all trials (lowest $t(13) = 3.43$, $p = .004$ on trial 1), and that group OT-C consumed more fluid than group O-C (lowest $t(12) = 2.77$, $p = .017$ on trial 1). The analysis also revealed no differences between groups OT-E and OT-C (largest $t(13) = 1.18$, $p = .256$ on trial 3), or between groups O-E and O-C (largest $t(12) = 1.72$, $p = .111$ on trial 3). The same ANOVA conducted with the lick cluster size data revealed an effect of trial, $F(3,75) = 40.75$, $p < .001$, and a significant trial x fluid interaction, $F(3,75) = 5.86$, $p = .001$. Importantly, there were no significant effects of fluid ($F<1$), or conditioning factors, $F(1,25) = 1.19$, $p = .286$. The interactions trial x conditioning, $F(3,75) = 1.345$ $p = .233$, fluid x conditioning, and the triple interaction ($Fs < 1$), were all not significant. The analysis of the trial x fluid interaction showed that groups OT-E and O-E did not differ each from the other (largest $t(13) = 0.93$, $p = .366$ on trial 2), and nor did groups OT-C and O-C also significant differ (largest $t(12) = 0.69$, $p = .499$ on trial 3). Also, the groups OT-E and OT-C did not differ (largest $t(13) = 1.59$, $p = .134$ on trial 2), nor did the groups O-E and O-C (largest $t(12) = 0.97$, $p = .350$ on trial 1). These results indicate

that there was no evidence of differential hedonic evaluation of the odor and the odor-taste compound during the pretraining phase despite differences in consumption.

The 2 (fluid: odor vs odor-taste) x 2 (conditioning: saccharin paired with LiCl vs saline) ANOVA performed with the consumption data from the conditioning session revealed no effects of fluid and conditioning, and no fluid x conditioning interaction (*Fs* < 1). The mean (± SEM) saccharin consumption (ml) for the different groups was: group OT-E: 12.91 (± 1.06); group O-E: 11.88 (± 1.34); group OT-C: 12.61 (± 0.49); group O-C: 13.65 (± 1.09). The statistical analysis conducted with the cluster size data also revealed no differences between the four groups. There were no main effects of fluid, $F(1,25) = 2.38$; $p = .135$, conditioning, $F(1,25) = 2.96$; $p = .098$, nor an interaction between these two factors, $F(1,25) = 1.22$; $p = .278$. The mean lick cluster size for each group was: group OT-E: 26.26 (± 1.76); group O-E: 32.11 (± 1.47); group OT-C: 24.92 (± 3.28); group O-C: 25.88 (± 1.93).

Fig. 1A (left-hand side) shows the mean consumption of the odor solution by the different groups during Test 1 (mediated aversion). As shown in the figure, rats in groups OT-E and O-E exhibited significantly less consumption than did rats in control groups. Importantly, rats which had prior odor-taste experience (group OT-E) showed a lower consumption than rats which were given the odor alone (group O-E). The 2 (fluid) x 2 (conditioning) ANOVA revealed significant main effects of fluid, $F(1,25) = 5.38$; $p = .029$, and conditioning, $F(1,25) = 98.85$; $p < .001$, but there was no interaction between these two factors, $F(1,25) = 2.41$; $p = .132$. Pairwise comparisons confirmed the critical result that Group OT-E showed significantly less consumption of the odor than Group O-E, $t(13) = 2.62$, $p = .021$. In addition, the LiCl-injected experimental groups O-E and OT-E consumed less of the odor than their respective controls O—C and OT-C (lowest $t(12) = 5.68$, $p < .001$ for the O-E vs O—C comparison).

Fig. 1A (right-hand side) shows the data from Test 2 (direct aversion) with the saccharin solution. It can be seen that animals injected with lithium, groups OT-E and O-E, displayed lower saccharin consumption than control groups, OT-C and O-C, reflecting effective conditioning of saccharin conditioning. The 2 × 2 ANOVA conducted with these data revealed a significant main effect of conditioning, $F(1,25) = 113.83$; $p < .001$, but no effect of fluid, nor a interaction between these factors (*Fs* < 1). The post hoc analysis confirmed that the LiCl-injected experimental groups O-E and OT-E consumed less saccharin than their respective controls O-C and OT-C (lowest $t(12) = 7.12$, $p < .001$ for the O-E vs O-C comparison). Importantly, groups OT-E and O-E did not differ each from the other ($t(13) = 0.24$, $p = .808$).

Fig. 1B shows the lick cluster size data from the test sessions with the odor (left-hand side) and the saccharin (right-hand side). The ANOVA conducted with the data from odor test revealed a significant main effect of the conditioning factor, $F(1,25) = 12.84$; $p < .001$, no effect of fluid, $F(1,25) = 1.58$, $p = .220$, and a significant interaction between these two factors $F(1,25) = 7.73$; $p = .010$. The analysis of the fluid x conditioning interaction with pairwise comparisons showed that group OT-E had a lower lick cluster size than either of groups OT-C or O-E (lowest $t(13) = 3.91$, $p = .002$ for the difference between groups OT-E and O-E). The analysis also showed that Group O-E did not differ from the group O-C, $t(12) = 1.22$, $p = .243$.

The ANOVA conducted with the data from the saccharin test revealed that the experimental groups, OT-E and O-E, had a lower cluster size than the control groups, indicating a decreased hedonic evaluation of the saccharin in the experimental groups. There was a significant effect of conditioning, $F(1,25) = 50.46$; $p < .001$, but no an effect of fluid $F(1,25) = 1.11$; $p = .300$, nor a interaction between these two factors, $F(1,25) = 2.76$; $p = .109$. The post hoc comparisons confirmed that the LiCl-injected experimental groups O-E and OT-E had lower lick cluster sizes for saccharin than their respective controls O-C and OT-C (lowest $t(13) = 3.21$, $p = .007$ for the OT-E vs OT-C comparison). The analysis also showed that groups OT-E and O-E did not differ between them, $t(13) = 1.22$, $p = .243$.

In conclusion, the results of Experiment 1 showed that when rats received odor-taste pairings prior to saccharin devaluation with LiCl, they exhibited both a reduction in odor consumption and lick cluster size, suggesting that the odor cue could acquire negative hedonic qualities through taste-mediated learning.

3.2. *Experiments 2a & 2b: orofacial reactivity test*

3.2.1. *Results of experiment 2a*

The mixed ANOVA performed with the consumption data from pretraining sessions revealed significant effects of the trial, $F(3108) = 35.25$, $p < .001$, and fluid factors, $F(1,36) = 8.40$, $p = .006$, and a significant interaction between them, $F(3108) = 9.13$, $p < .001$, but there was no effect of the conditioning factor (*F*< 1). The interactions trial x conditioning, fluid x conditioning, and the triple interaction were all not significant (largest $F(3108) = 1.82$, $p = 0.147$ for the trial x conditioning interaction). Post hoc analysis of the trial x fluid interaction with pairwise comparisons revealed that group OT-E showed higher consumption than group O-E on trials 2–4 (lowest $t(18) = 3.24$, $p = .005$ on trial 2), and that group OT-C consumed more fluid than group O-C (lowest $t(18) = 4.02$, $p = .001$ on trial 1). The analysis also revealed no significant differences between groups OT-E and OT-C (largest $t(18) = 1.67$, $p =$

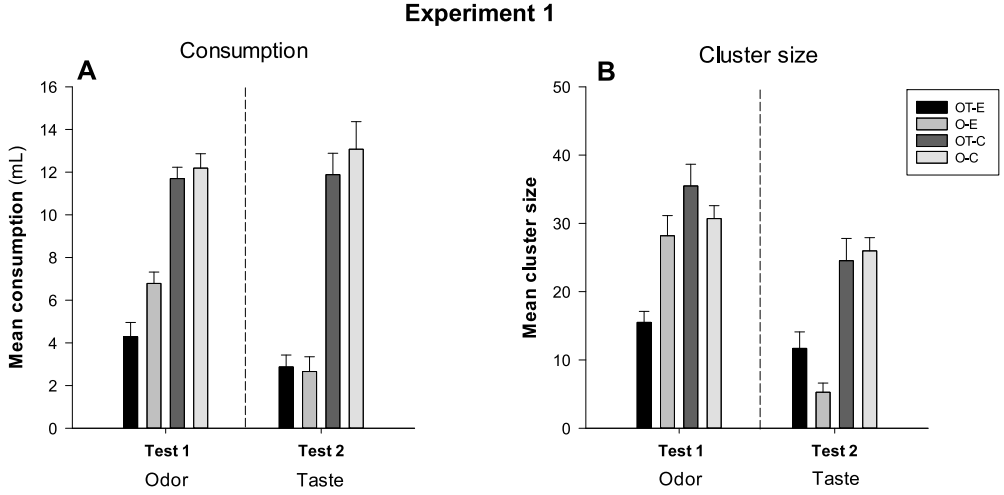

Fig. 1. Experiment 1. A) Mean odor solution intake (Test 1) and mean saccharin intake (Test 2) by the different groups during testing. B) Mean lick cluster size during Test 1 (odor) and Test 2 (saccharin). Error bars represent the standard error of mean (SEM).

.111 on trial 3), or between groups O-E and O-C (largest $t(18) = 1.65$, $p = .115$ on trial 3). The same ANOVA conducted with the lick cluster size data revealed a significant effect of trial, $F(3108) = 5.76$, $p = .001$, and a significant trial x fluid interaction, $F(3108) = 3.20$, $p = .026$, but there were not effects of the fluid and conditioning factors ($Fs < 1$). The interactions trial x conditioning, fluid x conditioning, and the triple interaction were all no significant (largest $F(3108) = 2.21$, $p = 0.09$ for the triple interaction). The analysis of the trial by fluid interaction with pairwise comparisons revealed that groups OT-E and O-E did not differ each from the other (largest $t(18) = 1.66$, $p = .113$ on trial 3), and that groups OT-C and O-C also did not differ between them (largest $t(18) = 0.98$, $p = .340$ on trial 3). Also, the groups OT-E and OT-C did not differ between them (largest $t(18) = 1.28$, $p = .215$ on trial 2), as well as the groups O-E and O-C (largest $t(18) = 672$, $p = .510$ on trial 4). These results indicate that the hedonic valuation of the odor and the odor-taste compound during pretraining was comparable despite the differences observed in consumption.

Table 2 presents the mean number of aversive orofacial responses displayed by the animals during the intraoral infusion of saccharin in the conditioning sessions. As shown in the table, there was a significant increase in the number of aversive responses across the trials in groups OT-E and O-E as compared with groups OT-C and O-C. A mixed ANOVA conducted with these scores revealed significant main effects of trial, $F(1,36) = 150.04$, $p < .001$, and conditioning, $F(1,36) = 147.32$, $p < .001$, and a significant trial x conditioning interaction, $F(1,36) = 189.03$, $p < .001$. There was no significant effect of the fluid ($F<1$), nor a significant trial x fluid interaction ($F<1$). The triple interaction was also not significant, $F(1,36) = 1.74$, $p = .195$. The post hoc exploration of the trial x conditioning interaction revealed that there were no significant differences between groups in Trial 1 (largest $t(18) = 1.25$, $p = .224$ for the difference between group O-E and O-C). However on Trial 2, the LiCl-injected experimental groups O-E and OT-E had displayed more aversive reactions than their respective controls O-C and OT-C (lowest $t(18) = 9.22$, $p < .001$ for the difference between groups O-C and O-E). The analysis also revealed no differences in Trial 2 between groups OT-E and O-E, or between groups OT-C and O—C (largest $t(18) = 11.68$, $p = .501$ for the comparison between groups OT-E and O-E). The comparisons between groups for Trial 1 were all not significant.

Table 2 also presents the mean duration (in seconds) of appetitive orofacial responses elicited by the infusion of saccharin during the conditioning trials. There was a significant reduction in appetitive responses in groups injected with lithium as compared with control groups. The ANOVA conducted with these data revealed significant main effects of trial, $F(1,36) = 240,14$, $p < .001$, and conditioning, $F(1,36) = 243,53$, $p < .001$, and a significant interaction between these two factors, $F(1,36) = 347,01$, $p < .001$, but not a significant effect of fluid ($F< 1$). The interactions involving trial and fluid, fluid and conditioning, and the triple interaction were all not significant (largest $F(1,36) = 2.85$, $p = 0.10$ for the trial x fluid interaction). There were no differences between groups in Trial 1 (largest $t(18) = 1,24$, $p = .229$ for the comparison between groups OT-E and OT-C). However, on Trial 2 post hoc pairwise comparisons revealed that both conditioned groups, OT-E and O-E, showed significantly fewer appetitive responses than their respective saline-injected controls OT-C and O-C (lowest $t(18) = 18,23$, $p < .001$ for the difference between groups O-E and O-C). The analysis also revealed no differences between groups OT-E and O-E, or between groups OT-C and O—C in Trial 2 (largest $t(18) = 1,27$, $p = .217$ for the difference between groups OT-C and O-C).

Table 2
Experiment 2a. Data from the conditioning phase: Mean number of aversive responses, and mean duration (in seconds) of appetitive responses elicited by the infusion of saccharin. Standard error of mean (*SEM*) is shown in brackets.

Group	Trial 1	Trial 2	Trial 1	Trial 2
	Aversives		Appetitives	
OT-E	1.20 (± 0.3)	18.20 (± 1.5)	65.96 (± 2.7)	8.97 (± 1.2)
O-E	0.90 (±0.3)	20.10 (± 2.1)	65.07 (± 3.4)	11.17 (± 1.4)
OT-C	0.80 (± 0.2)	0.60 (± 0.2)	70.72 (± 2.6)	71.86 (± 2.3)
O-C	2.20 (± 1.0)	0.30 (± 0.1)	68.04 (± 3.5)	77.07 (± 3.3)

Fig. 2A shows the mean number of aversive orofacial responses displayed by the different groups during Test 1 with the odor solution (left-hand side) and during Test 2 with the saccharin solution (right-hand side). As shown in the figure, rats in groups OT-E and O-E displayed more aversive responses when infused with the odor than did rats in control groups. Importantly, rats which had prior odor-taste experience (group OT-E) showed more aversive responses than rats which were given the odor alone (group O-E). The 2 (fluid) x 2 (conditioning) ANOVA revealed significant main effects of fluid, $F(1,36) = 10,46$; $p = .003$, and conditioning, $F(1,36) = 58,08$; $p < .001$, and a significant interaction between these two factors, $F(1,36) = 8,39$; $p = .006$. The analysis of the fluid x conditioning interaction with pairwise comparisons showed that group OT-E displayed more aversive responses during the odor infusion than either of groups O-E or OT-C (lowest $t(18) = 3.18$, $p = .005$ for the difference between groups OT-E and O-E), and that groups OT-C and O-C did not significantly differ from each other, $t(18) = 0.617$, $p = .545$.

A similar ANOVA conducted with the aversive responses displayed by the rats during saccharin infusion showed a significant effect of the conditioning factor, $F(1,36) = 29,38$; $p < .001$, but no effect of fluid ($F<1$), nor an interaction between these two factors $F(1,36) = 1.08$; $p = .305$. As shown in Fig. 2A (right-hand side), the groups OT-E and O-E displayed more aversive responses to saccharin than did the control groups, reflecting the fact that aversive conditioning was effective in reducing the saccharin hedonic evaluation. The post hoc comparisons confirmed that groups OT-E and O-E displayed more aversive responses to the saccharin than groups OT-C and O-C (lowest $t(18) = 3,37$, $p = .003$ for the difference between groups O-E and O-C). There were no differences between the groups OT-E and O-E ($t(18) = 1.03$, $p = .329$, or between the groups OT-C and O-C, $t(18) = 0.287$, $p = .777$.

Fig. 2B shows the mean duration of appetitive responses during the infusion of the odor (left-hand side) and saccharin (right-hand side). The groups OT-E and O-E displayed fewer appetitive responses to the odorant as compared with the control groups. The ANOVA conducted with these data revealed significant main effects of fluid, $F(1,36) = 5.33$; $p = 0.027$, and conditioning, $F(1,36) = 73.66$; $p < .001$, but there was no a significant interaction between these factors ($F < 1$). Post hoc comparisons confirmed that groups OT-E and O-E showed less appetitive responses than the control groups (lowest $t(18) = 6,06$, $p < .001$ for the difference between groups O-E and O-C), and that there were no differences between the groups OT-E and O-E, or between the groups OT-C and O-C (largest ($t(18) = 1.07$, $p = .296$ for the difference between the groups injected with lithium). The ANOVA performed with the data from the saccharin test showed a significant effect of conditioning, $F(1,36) = 162.87$; $p < .001$, but no an effect of fluid ($F<1$), nor an interaction between them, $F(1,36) = 1.06$; $p = .309$. As shown in the Fig. 2B (right-hand side), the groups OT-E and O-E displayed fewer appetitive responses than groups OT-C and O-C. The pairwise comparisons confirmed that the experimental groups displayed fewer appetitive responses than the control groups (lowest $t(18) = 7.84$, $p < .001$ for the comparison between the groups OT-E and OT-C), and that the experimental groups did not differ between them, $t(17) = 1,06$, $p = .304$.

In summary, this experiment confirmed the results obtained in Experiment 1 by using the orofacial reactivity test which provides a direct measure of the affective responses elicited by the odor cue. As in Experiment 1, it was found that odor cues can acquire negative hedonic qualities indirectly by taste-mediated learning.

3.2.2. Results of experiment 2b

The ANOVA conducted with the animal's consumption on odd days from pretraining (odor vs odor + taste) revealed significant effects of trial, $F(4,100) = 53.35$, $p < .001$, and group, $F(2,25) = 14.16$, $p < .001$,

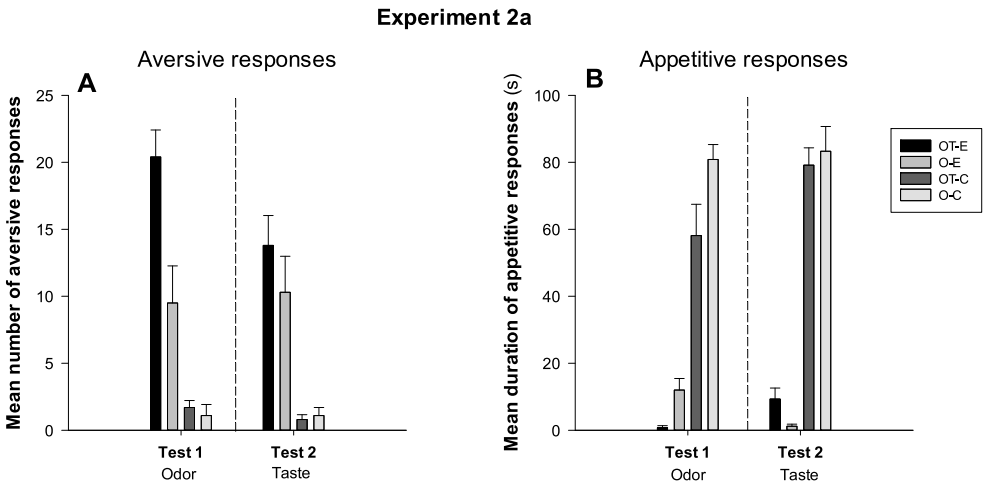

Fig. 2. Experiment 2a. A) Mean number of aversive orofacial responses displayed by the different groups during the intraoral infusions of odor (Test 1) and saccharin (Test 2). B) Mean duration (in seconds) of appetitive responses elicited by the infusions of odor (Test 1) and saccharin (Test 2). Error bars represent the standard error of mean (*SEM*).

and a significant interaction between them, $F(8,100) = 2.84$, $p = .007$. Post hoc analysis of the trial x group interaction revealed that group OT-E showed higher consumption than groups O/T-E and O/T-C on trials 2–5 (lowest $t(17) = 2.15$, $p = .045$ on trial 5, for the difference between groups OT-E and O/T-C). The analysis also revealed no differences between groups O/T-E and O/T-C (largest $t(16) = 0.77$, $p = .453$ on trial 1). A similar ANOVA conducted with the lick cluster size data revealed a significant effect of trial, $F(4,100) = 5.78$, $p < .001$, but no an effect of group, $F(2,25) = 0.77$, $p = .471$, nor a significant trial x group interaction ($F(8,100)) = 1.05$, $p = .404$. These results show that the hedonic valuation of the odor and the odor-taste mixture was comparable despite the differences observed in consumption.

The analysis performed with the consumption data on even days from pretraining (taste vs water) revealed significant effects of trial, $F(4,100) = 53.35$, $p < .001$, and group, $F(2,25) = 14.16$, $p < .001$, and a significant interaction between them, $F(8,100) = 2.84$, $p = .007$. Post hoc analysis of the trial x group interaction revealed that group OT-E showed higher consumption than groups O/T-E and O/T-C on trials 2–5 (lowest $t(17) = 2.15$, $p = .045$ on trial 5, for the difference between groups OT-E and O/T-C). The analysis also revealed no differences between groups O/T-E and O/T-C (largest $t(16) = 0.77$, $p = .453$ on trial 1). A similar ANOVA conducted with the lick cluster size data revealed a significant effect of trial, $F(4,100) = 5.78$, $p < .001$, but no an effect of group, $F(2,25) = 0.77$, $p = .471$, nor a significant trial x group interaction ($F(8,100)) = 1.05$, $p = .404$. These results show that the hedonic valuation of the odor and the odor-taste mixture was comparable despite the differences observed in consumption.

Data from the conditioning phase (aversive and appetitive orofacial responses) are showed in Table 3. There was an increase in the number of aversive responses elicited by the saccharin across the trials in groups OT-E and O/T-E compared with group O/T-C. The mixed ANOVA conducted with these scores revealed significant main effects of trial, $F(1,25) = 65.66$, $p < .001$, and group, $F(2,25) = 11.69$, $p < .001$, and a significant interactions between these two factors, $F(2,25) = 12.73$, $p < .001$. The post hoc analysis showed that groups which had received saccharin-LiCl pairings (OT-E and O/T-E) displayed significantly more aversive responses in Trial 2 than Group O/T-C (lowest $t(16) = 3.51$, $p = .003$ on trial 2, for the difference between the groups O/T-E and O/T-C). The analysis also revealed no differences in trials 1–2 between groups OT-E and O/T-E (largest $t(17) = 0.93$, $p = .364$ on trial 2). Regarding the appetitive responses elicited by the infusion of saccharin during conditioning, the statistical analysis showed significant effects of trial, $F(1,25) = 29.08$, $p < .001$, and group, $F(2,25) = 13.01$, $p < .001$, and a significant interactions between these two factors, $F(2,25) = 7.07$, $p = .004$. Post hoc comparisons revealed that the devalued groups showed significantly fewer appetitive responses in Trial 2 than the group injected with saline (lowest $t(16) = 4.40$, $p < .001$ for the difference between groups OT-E and O/T-C). The analysis also revealed no differences in trials 1–2 between groups OT-E and O/T-E (largest $t(17) = 0.37$, $p = .716$ on trial 1). There were no differences between groups in the first conditioning session (largest $t(16) = 1.38$, $p = .185$ on trial 1, for the comparison between groups O/T-E and O/T-C).

Fig. 3A displays the mean number of aversive orofacial responses elicited by the different groups during the infusion of the odor (Test 1) and the saccharin (Test 2) solutions. As shown in the figure, rats in Group OT-E elicited more aversive responses when infused with the odor compared to either group O/T-E or O/T-C. The one-way ANOVA conducted with these scores revealed a significant effect of group, $F(2,25) = 24.49$; $p < .001$. The pairwise comparisons confirmed that the odor solution elicited more aversive responses in the group OT-E than in groups O/T-E and O/T-C (lowest $t(17) = 4.91$; $p = 0.001$, for the comparison between groups OT-E and O/T-E), which did not differ between them ($t(16) = 0.63$; $p = 0.536$). The lack of differences between groups O/T-E and O/T-C confirms that the odor cue did not acquire aversive properties via generalization from the aversion to saccharin in the absence of prior odor-taste pairings. A similar ANOVA performed with the aversive responses to the saccharin solution showed a significant effect of group, $F(2,25) = 6,38$; $p = .006$. The post hoc pairwise comparisons revealed that the groups OT-E and O/T-E displayed more aversive responses than did the group O/T-C (lowest $t(17) = 3.39$; $p = 0.003$, for the comparison between groups O/T-E and O/T-C). The groups OT-E and O/T-E did not differ from each other, $t(17) = 0.26$; $p = 0.799$, reflecting the fact that aversive conditioning was effective in reducing the saccharin hedonic evaluation.

Fig. 3B shows the mean duration of the appetitive responses elicited by the infusion of the odor and the saccharin. As shown in the left-hand side of the figure, the groups OT-E and O/T-E displayed fewer appetitive

Table 3
Experiment 2b. Data from the conditioning phase: Mean number of aversive responses, and mean duration (in seconds) of appetitive responses elicited by the infusion of saccharin. Standard error of mean (*SEM*) is shown in brackets.

Group	Trial 1	Trial 2	Trial 1	Trial 2
	Aversives		Appetitives	
OT-E	0.40 (± 0.2)	21.70 (± 2.7)	40.87 (± 3.9)	6.95 (± 2.5)
O/T-E	0.55 (± 0.3)	17.22 (±4.1)	43.10 (±4.6)	6.60 (± 2.2)
O/T-C	0.51 (± 2.3)	2.44 (± 0.9)	57.79 (± 5.6)	57.65 (± 5.4)

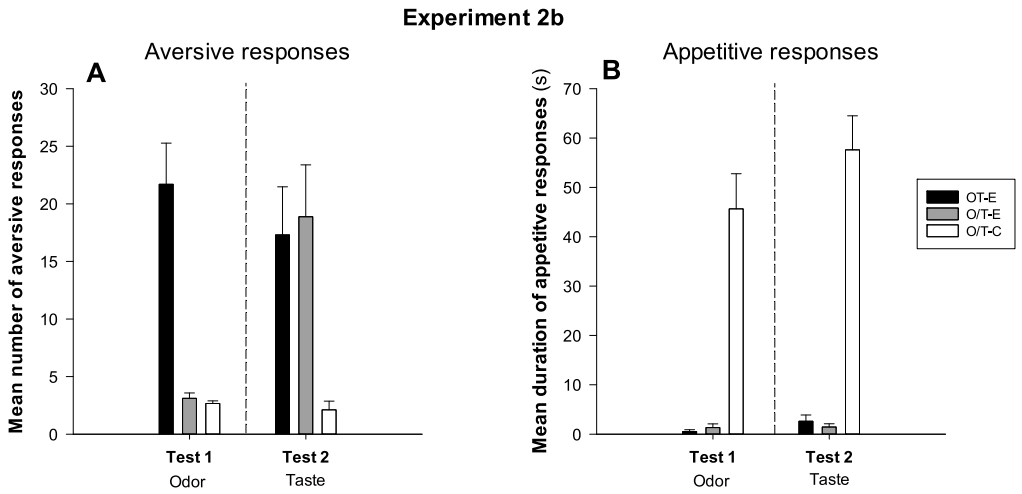

Fig. 3. Experiment 2b. A) Mean number of aversive orofacial responses displayed by the different groups during the intraoral infusions of odor (Test 1) and saccharin (Test 2). B) Mean duration (in seconds) of appetitive responses elicited by the infusions of odor (Test 1) and saccharin (Test 2). Error bars represent the standard error of mean (SEM).

responses when infused with the odor than group O/T-C. The ANOVA conducted with these scores revealed a significant effect of group, $F(2,25) = 17.43$; $p < 0.001$. Post hoc comparisons confirmed that group O/T-C showed more appetitive response than groups OT-E and O/T-E (lowest $t(16) = 4.01$; $p = .001$, for the comparison between groups O/T-E and O/T-C). The groups OT-E and O/T-E did not differ between them, $t(17) = 0.97$; $p = 0.341$. As for the data from the saccharin test (Fig. 3B, right-hand side), the ANOVA also revealed a significant effect of group, $F(2,25) = 41.55$; $p < 0.001$. The post hoc analysis showed that groups OT-E and O/T-E displayed less appetitive responses than the group O/T-C (lowest $t(17) = 6.41$, $p < .001$ for the difference between groups O/T-E and O/T-C), and that there were no differences between the groups OT-E and O/T-E, $t(17) = 0.77$; $p = .448$.

In summary, the present experiment replicated the results obtained in Experiment 2a showing the acquisition of affective responses by odor cues through taste-mediated learning in animals having had prior odor-taste pairings. In addition, the results argue against the possibility that the odor cue acquires conditioned properties by generalization from the conditioned aversion to the devalued taste in the absence of prior experience of the odor and taste together.

4. Discussion

The three experiments reported here examined the affective responses conditioned to odor cues in the taste-mediated odor aversion learning paradigm. Analyzing the microstructure of licking behavior, we found in Experiment 1 that saccharin devaluation with LiCl after odor-saccharin pairings resulted in both reduced intake of the odor solution and reduced lick cluster size, results indicating a reduced hedonic evaluation of the odor cue. Experiments 2a and 2b confirmed this result by examining the orofacial reactivity responses elicited by the infusion of the odor after saccharin devaluation. It resulted in an increase in the number of aversive orofacial responses elicited by the odor in rats that had prior odor-saccharin experience as compared with subjects receiving the odorant alone, a result again indicating a change in the hedonic value of the odor cue from positive to negative.[6]

One potential problem with the above conclusion is the fact that we also observed decreased consumption (Experiment 1) and increased orofacial aversive responses (Experiment 2a) in rats receiving exposures to the odor alone prior to the saccharin devaluation. As noted above, it may be that the odor cue acquires conditioned aversive properties via generalization of the aversion conditioned to the saccharin because this solution has both taste and odor components which are presumably available for conditioning. Two findings argue against this explanation: In Experiment 1 it there was a reduction in odor consumption but, importantly, no decrease in lick cluster size in rats having experience with the odor alone prior to the devaluation of the saccharin; and Experiment 2b replicated the results of Experiment 2a, finding increased aversive responses to the odor in rats that had odor-taste pairings prior to the saccharin devaluation, but not in rats that had prior experience with the odor and the taste separately. In our view, experiencing the odor and the taste separately should attenuate any generalization to the odor cue of the conditioned properties, including hedonic responses acquired by the gustatory cue. Taken together, these results provide evidence of conditioned changes in affective value of odor cues.

Thus, there is clear evidence that after initial odor-saccharin pairings, devaluation of the saccharin taste with LiCl-induced illness also results in a reduction in the hedonic value of the odor. There are several possible associative mechanisms for this effect: A – Representation-mediated conditioning as initially described by Holland [22,23,30]; B – Sensory preconditioning as a chain of associations (for a review see [31]); C – Generalization between saccharin and the odor due to the odor acquiring sweet-taste properties [5,32].

Taking these in turn, Holland's analysis of mediated conditioning was that after the pairing of two cues, presentation of one could retrieve the representation of the other, and this retrieved representation might support excitatory conditioning if a US was presented at the same time. In the current procedures, this would suggest that during saccharin-LiCl pairings, saccharin could have retrieved the representation of the odor, and in turn, this retrieved representation of the odor would be associated with LiCl-induced illness. The idea of sensory preconditioning as a chain of associations is also based on the assumption that saccharin-odor pairings produce associations between the two, which allow one to retrieve the representation of the other. But in this case the assumption is that the effect is determined at the test phase: here, it would be assuming that the odor retrieves the representation of saccharin, and in turn the retrieved representation of saccharin would activate the memory of LiCl-induced illness. However, while both mediated conditioning and sensory preconditioning are logically possible accounts of

[6] There was also a non-specific effect in Experiments 2a and 2b whereby appetitive reactions low in all animals treated with LiCl when tested with the odor. This may reflect a general suppression of appetitive responses after experience of LiCl-induced illness, or perhaps context-based suppression of responding given that the saccharin-LiCl pairings occurred in the same context as the test phase.

the effects observed here, it should be noted that studies of both mediated conditioning [22,23,30] and sensory preconditioning with taste stimuli [33,34] are typically reported to be smaller effects than is seen when a taste (or odor for that matter) is directly paired with LiCl. In the current studies the indirectly conditioned odor aversion, as indexed by consumption and lick cluster size (Experiment 1) or orofacial reactivity (Experiments 2a/2b) is of comparable size to the directly conditioned saccharin aversion. Thus, neither options A nor B appears to be a close fit with the observed data.[7] In contrast, if the phase 1 odor-saccharin pairings resulted in the odor acquiring the sweet properties of saccharin, then generalization from saccharin to the (now sweet) odor could produce learning about the odor during the conditioning phase, and/or allow the odor to the memory of LiCl-induced illness via the sweet taste of saccharin at test [5,32]. This generalization mechanism is less well characterized than either mediated conditioning or sensory preconditioning, but if the degree of generalization is high, then it would in principle support equivalent responses to the odor and saccharin. Thus, the generalization account seems to be the most consistent with the current data, and this should motivate additional consideration of the mechanisms by which such acquired generalization operates.

Putting to one side the exact associative mechanisms underpinning the current effects, it should be remembered that different behavioral tasks have been employed to examine odor perception and the hedonic qualities of odorants in rodents, including olfactory discrimination [7], odor-cued taste avoidance [35], and odor preference conditioning [8]. In these procedures the odor hedonic value is inferred from smelling time directed towards the odor, the licking rate from a drinking bottle containing the odorant, the amount of the odorous solution ingested, or changes in preference for an odor previously paired with a sweet taste. However, it is known from taste aversion studies that some treatments produce a reduction in voluntary consumption that is not accompanied by a reduction in the hedonic value of the taste. For example, pairing saccharin with events having aversive consequences (e.g., footshock, injections of hypertonic saline, and some drugs of abuse such as amphetamine) results in suppressed consumption of the saccharin but not in the production of aversive orofacial responses in the taste reactivity test indicative of a reduction in its affective value [27,36]. Thus, voluntary consumption is not a selective measure of conditioned hedonic responses. The present experiments examining the microstructure of licking behavior and orofacial responses provide direct evidence of the affective responses conditioned to the odor. One important implication of the present study is that it shows that odor stimuli appear to engage the same processes as taste cues in aversion learning. Recent work from our laboratory have demonstrated that contextual, non-flavor, cues paired with nausea produced by LiCl injections can elicit aversive orofacial responses as do LiCl-paired flavors, suggesting that aversion learning is governed by general associative mechanisms [24,37]. We have also demonstrated that flavors paired with nausea or with internal pain produced by hypertonic saline elicit divergent types of hedonic response: Only pairing with nausea results in the production of aversive orofacial responses to the taste whereas pairing with internal pain results in the taste eliciting immobility (reflecting fear), despite equivalent reductions on flavor consumption [24]. Taken together, these studies suggest that the quality of aversion learning, including the affective responses elicited by the fluids, is primarily determined by the nature of the aversive event (nausea, pain) and not the type of conditioned cue (taste, odor, context).

As mentioned earlier, flavor perception is a multisensory experience involving the integration of different properties of foods and fluids, including their hedonic qualities. Indeed, a number of studies in human and animal models provide evidence of neural representation of odor hedonics (and olfactory-taste convergence) in the gustatory cortex, orbitofrontal cortex, piriform cortex, and at different levels of the olfactory system [38–41]. For example, work examining c-Fos activity has identified two critical regions involved in processing of gustatory and olfactory information, the gustatory and the piriform cortex, respectively. Specifically, it has been found that novel tastes and novel odors, or novel odor-taste mixtures, elicit greater c-Fos activity in the gustatory cortex compared with animals having prior experience with such solutions. However, increased c-Fos expression in the piriform cortex is observed only with novel solutions containing odors [42]. These results support the idea that gustatory cortex is a fundamental brain area for the integration of gustatory and olfactory signals [43–46].

Finally, the importance of associative learning in flavor perception and, particularly in the integration of olfactory and gustatory information of foods and fluids, should be emphasized. There is some evidence that odors can even acquire taste-like qualities when the odor is repeatedly experienced with tastes [5,32,43]. However, as noted above, in these studies the gustatory properties acquired by odors are inferred from consumption tests, and do not provide a direct evidence of the affective responses after pairing with flavors. To provide a better understanding of functional integration of olfactory and gustatory information in flavor learning, future studies should focus on the behavioral and neural mechanisms involved in odor and taste hedonics.

CRediT authorship contribution statement

Matías López: Methodology, Formal analysis, Data curation, Funding acquisition, Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Dominic M. Dwyer:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **Patricia Gasalla:** . **Azucena Begega:** Funding acquisition, Investigation. **Claudia Jove:** Data curation, Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare no competing financial and non-financial interests.

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