bio-protocol

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Characterizing Hedonic Responses to Flavors Paired with Internal Pain and Nausea through the Taste Reactivity Test in Rats

Matías López[1, *, #], Dominic M. Dwyer[2, #], Patricia Gasalla[2, #], Claudia Jove[1, #] and Azucena Begega[1, #]

[1]Department of Psychology, University of Oviedo, Oviedo, Spain
[2]School of Psychology, Cardiff University, Cardiff, UK
*For correspondence: mlopez@uniovi.es
[#]Contributed equally to this work

Abstract

Feeding behavior is a complex experience that involves not only sensory (i.e., visual, odor, taste, or texture) but also affective or emotional aspects (i.e., pleasure, palatability, or hedonic value) of foods. As such, behavioral tests that assess the hedonic impact of foods are necessary to fully understand the factors involved in ingestive behavior. In this protocol, we use the taste reactivity (TR) test to characterize the hedonic responses of rats to flavors paired with either lithium chloride–induced nausea or internal pain produced by hypertonic NaCl, two treatments that reduce voluntary consumption. This application of the TR test demonstrates how emetic and non-emetic (somatic pain in particular) treatments produce dissociable patterns of hedonic reactions to fluids: only emetic treatments result in the production of aversive orofacial responses, reflecting conditioned nausea, whereas somatic pain produces immobility, reflecting conditioned fear. Other methods, such as the microstructural analysis of licking behavior, do not reliably distinguish conditioned nausea and fear, a key advantage of the more selective TR procedure. This protocol also contains guidance for adaptation to other species and designs.

Keywords: Taste aversion, Hedonic responses, Orofacial reactivity, Nausea, Internal pain, Rats

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Background

In rats, pairing a novel flavored solution with nausea produced by the administration of emetic drugs such as lithium chloride (LiCl) results in the subsequent reduction in consumption of that flavor, a learning phenomenon termed conditioned taste aversion (CTA) (see Reilly and Schachtman, 2009 for a review). Critically, not only does CTA result in decreased consumption of that flavor, but there is also evidence of a change in its palatability or hedonic qualities, as suggested by John Garcia, the pioneer in studying this learning phenomenon (Garcia et al., 1955). However, CTA is not exclusively produced by emetic treatments. Pairing flavors with a wide variety of other aversive events, including pain produced by footshock or injection of hypertonic saline, as well as the administration of many drugs of abuse, reliably produces reductions in voluntary consumption (e.g., Pelchat et al., 1983; Parker, 1995; Arthurs et al., 2012). Thus, one central issue in this protocol is whether emetic and non-emetic treatments produce the same sorts of behavioral affective reactions to fluids. The taste reactivity (TR) protocol we describe here can be generalized for many uses, including determining whether a novel substance or intervention is aversive because it produces pain or nausea (and similarly whether a treatment ameliorates pain/nausea). Additionally, this procedure is amenable to be adapted for studying the neural mechanisms of hedonic evaluation (Inui and Simura, 2017), or for examining the role of anhedonia in psychiatric and neurological disorders such as depression and schizophrenia (Ward et al., 2012).

The optimal method affording a direct assessment of the hedonic impact of flavors is the TR test (Grill and Norgren, 1978). This involves examining orofacial reactions—stereotyped oromotor and somatic consummatory responses—elicited during the intraoral infusion of the fluid in the rat's oral cavity. These orofacial responses can be classified as aversive (e.g., gaping, chin rubbing, and paw treading), elicited when infused with unpleasant sour or bitter tastes, or appetitive (e.g., tongue protrusions, mouth movements, and paw licks), elicited by pleasant, sweet tastes. When infused with a palatable taste (e.g., saccharin) previously paired with LiCl-induced nausea, rats display aversive responses, reflecting a shift in the hedonic value of the flavor from positive to negative. Importantly, this technique is selective to disgust, in contrast to the reduction in consumption that may simply reflect taste avoidance without a change in affective responses. As mentioned above, pairing a flavor with peripheral pain or with rewarding drugs, such as cocaine or amphetamine, results in a reduction in subsequent voluntary consumption of that flavor, but not in the production of orofacial aversive responses (Parker, 1995). This dissociation in the impact of emetic drugs and other aversive events supports the suggestion that a reduction in fluid intake may be motivated by two different processes: a taste associated with nausea causes a reduction in its palatability (CTA), whereas a taste associated with a drug of abuse or peripheral pain is avoided because it signals a potential danger or a disturbance in homeostasis regulation [taste avoidance learning (TAL)] (see Parker 2003, 2014).

The hedonic value of flavors can also be examined by analyzing the microstructure of licking behavior during voluntary consumption (Davis, 1989; Dwyer, 2012). The ingestive behavior of rodents consuming fluids consists of sustained runs of licks separated by pauses of varying length (clusters); 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, decreasing monotonically with an increased concentration of unpalatable quinine solutions. In the context of taste aversion learning, pairing an otherwise palatable taste with nausea results in a reduction of lick cluster size, similar to that produced by exposure to quinine. However, reductions in cluster size cannot be unambiguously attributed to conditioned disgust (for example, these do not distinguish between the effects of hypertonic saline and isotonic lithium chloride as described here) in the absence of careful observation of orofacial behavioral reactions directly indicative of a particular emotional response. With this issue in mind, here we use the orofacial reactivity protocol to selectively characterize hedonic responses to flavors paired with either nausea or internal pain caused by injection of hypertonic saline.

Materials and Reagents

1. PE-160 intramedic polyethylene tubes (Becton Dickinson, MD, USA, catalog number: 427431) attached to an infusion pump, for intraoral infusion
2. 15-gauge stainless steel needles (Kruuse, Denmark, catalog number: 120753), to implant intraoral cannula

3. Microlance 3 25 g × 5/8" hypodermic needles (Becton Dickinson, MD, USA, catalog number: 300600), for injection procedures
4. Animals: Wistar rats (280–393 g, male, 9 weeks old; University of Oviedo vivarium, Spain)
5. Fluids used for intraperitoneal injections: LiCl (0.15 M,10 mL/kg) (VWR Chemicals, catalog number: 25007-230), NaCl (1.5 M, 10 mL/kg), and isotonic saline (0.9%,10 mL/kg) (VWR Chemicals, catalog number 27810-262)
6. Flavor used for intraoral infusion: 0.1% (w/w) saccharin sodium (Sigma-Aldrich, Merck, catalog number: 47839)
7. Ketamine hydrochloride (50 mg/kg) (Sigma-Aldrich, Merck, catalog number: 1356009)
8. Medetomidine hydrochloride (0.15 mg/kg) (Sigma-Aldrich, Merck, catalog number: 1179333)
9. Ketoprofen (1.5 mg/kg) (Sigma-Aldrich, Merck, catalog number: 1356632)
10. Enrofloxacin (0.3 mg/kg) (Sigma-Aldrich, Merck, catalog number: 17849)
11. Chlorhexidine (0.3 mg/kg) (Sigma-Aldrich, Merck, catalog number: 282227)
12. PE-90 intramedic polyethylene tubes (Becton Dickinson, MD, USA, catalog number: 427519) for intraoral cannula
13. O-ring Mini Stix ligature ties (TP Orthodontics Inc., IN, USA, catalog number: 383-927), to hold securely the PE-90 tubes in the oral cavity
14. Injection syringes 1 mL (Becton Dickinson, catalog number: 309628) and 5 mL (BD, catalog number: 309646)

Equipment

1. Custom-made TR apparatus (see Figure 1)
2. Infusion pump (KD Scientific Inc, MA)
3. Video camera (Sony Optical 20×) connected to a desktop computer

Figure 1. Taste reactivity apparatus.
Front view of the TR apparatus with the different components. (A) Infusion chamber. (B) Infusion pump. (C) Mirror at a 45° angle. (D) Video camera. (E) Computer. See Note 2 for a more detailed description of the apparatus.

Software

1. The Observer XT 9.0 (Noldus Information Technology, Sterling, VA) event-recording program, to score videos. This computer software is an automated system that makes it possible to record the behavior of animals directly in the TR chamber and code the sequence and duration of the behaviors exhibited in a quantitative way.

Procedure

See Figure 2 for a schema depicting the different steps of the protocol.

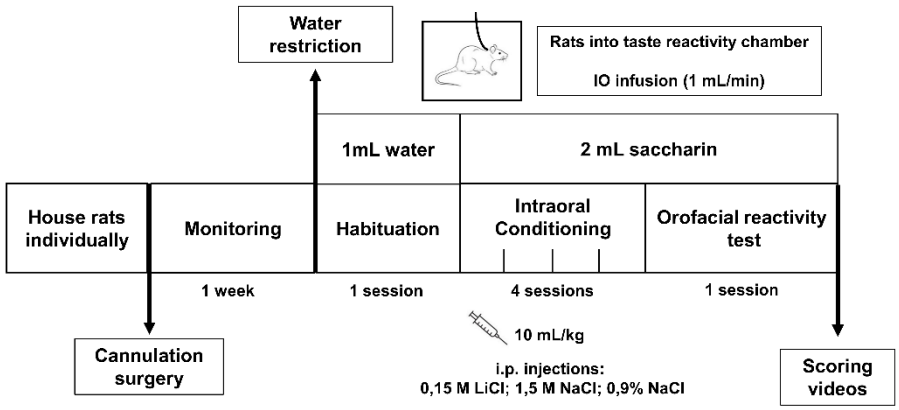

Figure 2. Behavioral protocol to investigate hedonic responses using the TR test.

1. House rats individually in standard (e.g., 42 × 26 × 20 cm) polycarbonate cages in a colony room under controlled standard conditions (12 h light/dark cycles starting at 8 am; ambient temperature of 21 °C). Always make food available in the home cages. Note that individual housing is to prevent rats from pulling out cannula from cage mates.
2. Cannulation surgery
   Surgically implant rats with an intraoral cannula using the method described by Parker (1995) and as previously used in our laboratory (Gasalla et al., 2016, 2017). The cannulation procedure (see Figure 3) is described in detail in Note 4 below.
3. Monitoring and habituation
   After recovery from the surgery, place rats on a water-restriction schedule, comprising 1 h daily access to water (given approximately 2 h after any experimental fluid exposure) and free access to food in the home cage. Home cage food and water intake can be monitored, but this is not essential. Then, habituate rats to the infusion procedure by delivering a 1 mL water infusion (rate of infusion 1 mL/min) in the TR apparatus.
4. Intraoral conditioning
   The next four days constitute the conditioning phase. On these sessions, place rats in the TR apparatus (with their cannula attached to the infusion pump for fluid delivery). Intraorally infuse them with the saccharin solution for 2 min (1 mL/min) and deliver an injection of LiCl (group Lithium), hypertonic NaCl (group Hypertonic), or isotonic saline (group Isotonic), immediately after the intraoral infusion. Give the rats water-recovery days in the home cage after the second and fourth conditioning sessions. Video-record the orofacial responses displayed by the rats during the infusion of the saccharin solution.
5. Orofacial reactivity test
   Perform a TR test the day after the last conditioning session. Deliver an intraoral administration of the saccharin solution for 2 min (1 mL/min) while video-recording the rats' orofacial responses.

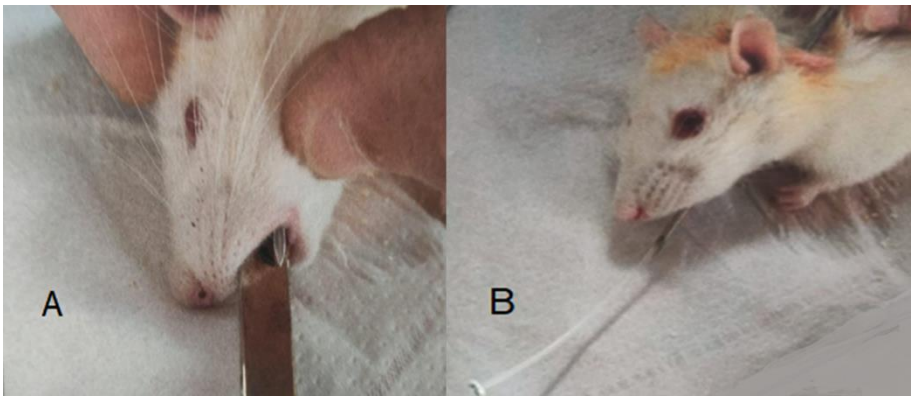

Figure 3. Cannulation procedure.
The figure shows two steps of the cannulation procedure. (A) Insert a guide needle inside the mouth. (B) Insert the intraoral cannula through the needle. See Note 4 for a more detailed description of the cannulation procedure.

Scored orofacial reactions:

1. Appetitive responses: tongue protrusions (extension of the tongue out of 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). Use the total number of seconds that the rats display the responses as the appetitive response score. For representative appetitive TR responses, see Figure 4A and Video 1 showing orofacial responses scored in this protocol.
2. Aversive responses: 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), paw treading (forward and backward movements of the forepaws in synchronous alternation), forelimb flails (rapid horizontal movements of the forelimbs to 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). Sum these scores to provide a total aversive response score (see Figure 4B and Video 1 for representative aversive responses).
3. Assess the percentage of time spent immobile over the infusion period (scored as suppression of all movements in the rat, with the exception of those required for respiration) as an index of conditioned fear. In addition, score the frequency of passive dripping (each occasion on which a drop of fluid is allowed to leak out of the mouth to the floor without other orofacial reactions; see Figure 4C and Video 1).

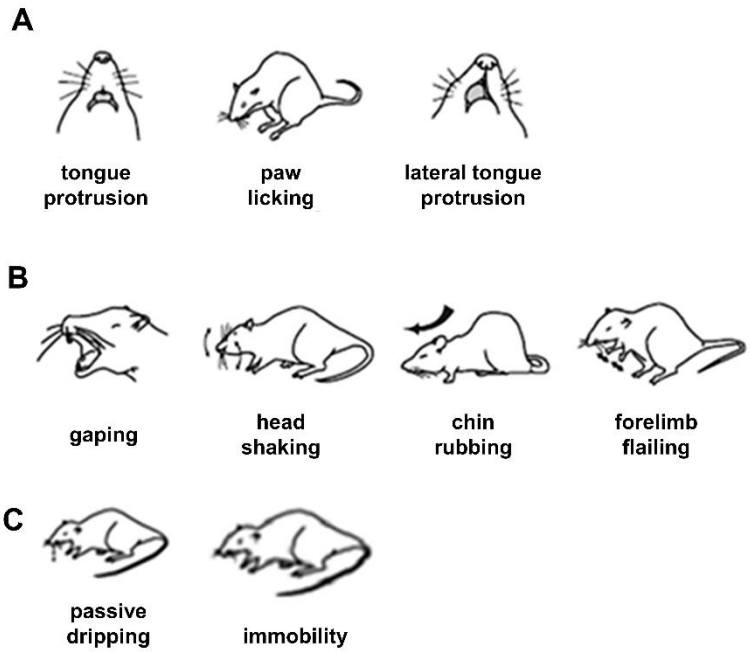

Figure 4. Representative orofacial reactivity responses.
(A) Appetitive responses: tongue protrusion, paw licking, and lateral tongue protrusions. (B) Aversive responses: gaping, head shaking, chin rubbing, and forelimb flailing. (C) Passive responses: dripping and immobility (Based on Inui and Shimura, 2017).

Video 1. Orofacial responses video-recorded during the intraoral infusion of the flavor.
After completion of the behavioral procedures, score the videos using the Observer XT 9.0 event-recording program.

Data analysis

Separately analyze the responses (appetitive, aversive, passive dripping, and immobility) scored during the conditioning sessions and test with 3 (group) × 5 (session) mixed ANOVAs. Detailed information on data analyses appears in the original research article (Dwyer et al., 2017). Figure 5 shows representative results obtained in the research. Pairing a taste with either LiCl-induced nausea or internal pain reduced appetitive responses, but these two aversive events had clearly dissociable effects on other responses: 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 and passive dripping. In addition, both nausea and internal pain reduced voluntary consumption of the paired flavor (data not shown).

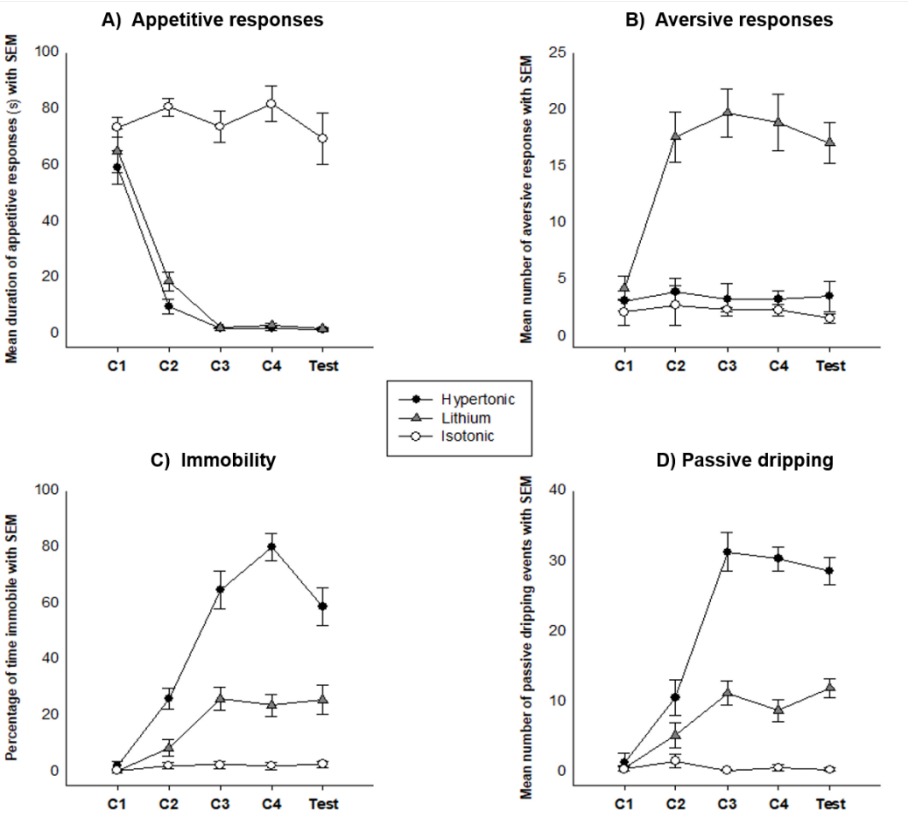

Figure 5. Representative results of the intraoral conditioning (C1-C4) and test sessions.
(A) Mean duration of appetitive orofacial responses. (B) Mean number of aversive orofacial responses. (C) Mean time spent immobile as a percentage of the total time tested. (D) Mean number of passive dripping events (adapted from Dwyer et al., 2017). Error bars represent the standard error of mean (SEM).

Notes

1. This protocol is designed for rats as subjects but is suitable for use with other rodents, including outbred mice (e.g., Kiefer et al., 1998), transgenic mice (e.g., Travers et al., 2007), and shrews (e.g., Parker, 2006).
2. The TR apparatus is made of clear Plexiglas sides (26 × 23 × 14 cm) with a dark lid, placed on a table with a clear Plexiglas top. Two 50 W white lights on each side of the table provided illumination. A mirror beneath the chamber at a 45° angle facilitated viewing/filming of the ventral surface of the rat during the intraoral infusion. Recording from this angle is essential to unambiguously observe the orofacial reactions, which can otherwise be obscured if the recording is from the side or above. As shown in Video 1, the recording should frame the head region of the animal as closely as possible.
3. For the purpose of anesthesia, the rats are intraperitoneally injected with ketamine (50 mg/kg) combined with medetomidine hydrochloride (0.15 mg/kg). After surgery, the rats are subcutaneously injected with the anti-inflammatory ketoprofen (1.5 mg/kg) and the antibiotic enrofloxacin (0.3 mg/kg).
4. In order to implant the cannula, a thin-walled 15-gauge stainless steel needle is inserted at the back of the neck, subcutaneously directly around the ear, and brought out behind the first molar inside the mouth. A length of

Iintramedic polyethylene tube with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm is then run through the needle, after which the needle is removed. Two square elastic discs are placed over the tubing and drawn to the exposed skin at the back of the neck with the purpose of stabilizing the cannula. The tubing is held secure in the oral cavity by an O-ring, which is sealed behind the tubing prior to cannulation surgery. Following surgery, rats are monitored for three days and have their cannula flushed daily with chlorhexidine (0.3 mg/kg) to prevent infection.

5. Fluids are administered to the rats through an infusion pump connected to the implanted cannula. While the rats are infused with the fluids, their orofacial responses are recorded using a video camera connected to a computer. Later, the videos are manually scored by two independent raters blind to the experiment using the event-recording program. An inter-rater reliability score for each response of at least 75% is deemed acceptable.
6. In the current protocol, appetitive and aversive responses are scored on different scales (duration vs. frequency) because they display very different properties: appetitive responses are typically displayed over extended periods of time, whereas aversive responses occur as isolated behaviors (see Berridge, 2000). Passive dripping and immobility are scored exclusively, such that time spent dripping is not also recorded as immobile. The different scales on which the responses are scored require each to be analyzed separately (as in the example described above). The TR analysis often uses a subset of the reactions (in particular, the “strong aversive” reactions of gaping, chin rubbing, and paw treading) to focus on clear aversive responses.
7. Parameters such as drug doses used for anesthesia and cannulation surgery, the volume of fluids administered, or the infusion rate during intraoral conditioning and testing are specific to the current experimental protocol. These procedural details may vary according to factors such as the weight of the rats or the species of rodent used in the study. Above, we specify an infusion rate of 1 mL/min over a period of 2 min. Higher infusion rates can be aversive due to the involuntary exposure to fluids at a rate higher than voluntary ingestion. Lower infusion rates are possible, although rates below 0.5 mL/min can result in insufficient solution exposure to elicit orofacial reactions. Longer infusion times are possible; however, infusion periods longer than 5 min tend to result in an increase in unconditioned passive and aversive responses. The use of multiple exposure sessions in the current protocol is required to track the acquisition of conditioned nausea and fear. When the TR test is used to probe unconditioned reactions (e.g., to palatable sucrose vs. unpalatable quinine), a single session per solution may be sufficient (especially if the exposure period is lengthened beyond the 2 min specified above).
8. As noted above, some procedural details of the TR method can be adapted for other species of rodents, in particular parameters such as the infusion rate and the amount of fluid infused. In mice and shrews, the infusion rate is usually 0.1 mL over a period of 1 min. Longer infusion rates generally induce more aversive reactions to sweet and bitter taste solutions in these rodents (Cagniard and Murphy, 2009).

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Competing interests

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

Ethics

This experimental protocol followed the current guidelines of the European Council Directive (210/63/UE) and

Spanish regulation RD53/2013 regarding the care and use of laboratory animals.

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