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Palatability changes during attenuation of flavor neophobia assessed with licking microstructure and taste reactivity

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ABSTRACT

Novel flavors elicit a neophobic response, which attenuates as they are recognized as safe. The attenuation of taste neophobia has been widely studied through measuring overall intake, however, changes in hedonic value have not been systematically considered. We conducted two experiments to assess, in both male and female Sprague-Dawley rats, the hedonic changes across a six-day attenuation of the neophobia process using two different methods. In the first experiment, we analyzed the microstructure of licking, which affords measuring both consumption and hedonic value at the same time. This study used animals heterozygous for *Cacna1c* (a key genetic risk factor for multiple psychiatric disorders associated with anhedonia) and wild type controls. In the second experiment, we used the taste reactivity test. The analysis of licking microstructure, but not the taste reactivity test, was sensitive to hedonic value changes during attenuation of the neophobia. Females showed more hedonic reactivity than males in both experiments, and there was no effect of the *Cacna1c* manipulation. The findings highlight the importance of considering hedonic factors in the attenuation of neophobia process. The difference between the hedonic assessment methods in the sensitivity to hedonic changes during attenuation of neophobia is discussed, although this is complicated by differences in the procedures themselves (e.g. voluntary vs involuntary consumption).

1. Introduction

Recognizing taste novelty and evaluating the consequences of ingesting novel foods play critical roles in diet selection and survival in omnivorous species such as humans and rodents (Gallo, 2018). Rodents exhibit taste neophobia, that is, they are reluctant to ingest novel flavors. In particular, they consume low amounts on the first presentation and if no aversive consequences occur following initial ingestion, intake increases as the flavor becomes familiar across later exposures until reaching an asymptote. This process is termed attenuation of neophobia (AN). Alternatively, if consumption leads to illness, conditioned taste aversion (CTA) takes place and the flavor will be avoided (Garcia et al., 1955, 1968; Revusky & Bedarf, 1967). Subsequent exposure to the aversive flavor without consequences leads to the extinction of the aversive conditioned response. In contrast, conditioned flavor preferences (CFP) can be developed if consumption is followed by positive consequences, such as nutrients (Elizalde & Sclafani, 1988; Riordan & Dwyer, 2019). Thus, when examining consumption only through overall intake it can be difficult to distinguish the processes involved in AN, CTA extinction and CFP. Indeed, while the observation of increased consumption after initial neophobia *is* the behavioral effect referred to as AN, examination of contributions from learning potentially speak to the mechanisms underlying that effect.

In this light, it is worth noting that AN experiments usually involve water deprived rodents, with water restriction implemented to motivate at least some consumption of the unfamiliar (and typically unpleasant) test flavor. As such, flavors will be followed by positive consequences in thirsty animals, and in turn there will be potentially competing influences – unfamiliarity and neophobia acting against consumption, the positive post-ingestive consequences promoting consumption. That said,

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neophobia and its attenuation occurs in a similar way in water deprived and non-deprived animals (Domjan, 1976), with the main variable determining increased intake across AN being the number of times the animal was previously exposed to the flavor. On balance, given water deprivation does not influence whether neophobia or AN occurs, it is typically preferable to deprive the animals to ensure at least some voluntary consumption of the unfamiliar flavor. But the need for caution in interpreting the mechanisms of AN given the possibility for conditioned preference based on positive consequences of fluid remains.

While most studies of AN have focused on consumption as the primary measure, it is important to recognize overall intake is an "endpoint" measure susceptible to multiple potential influences. In general, likes and dislikes modulate approach and avoidance behaviors, impacting on both the overall amount consumed, and the manner of consumption. However, overall solution intake is influenced by a number of factors beyond hedonic reactions, including motivational, motor functions (Dwyer, 2012) satiation state (Peck & Ader, 1974) and pain or illness (Dwyer, 2012; Garcia & Koelling, 1966; Peck & Ader, 1974). In addition, the importance of distinguishing between "wanting" and "liking" has been emphasized (Berridge, 1996; Morales & Berridge, 2020). In Berridge's terms, "wanting" refers to a motivational state related to incentive salience, while "liking" refers to the hedonic value or palatability of the flavor. Thus, a distinction has been proposed between learning procedures that modify the rewarding properties of flavors and those altering the hedonic responses, be they either appetitive or aversive. One example of this sort of distinction with respect to learned aversive responses has been between conditioned taste *avoidance* and conditioned taste *aversions* (Parker, 2003, 2014; Parker & Limebeer, 2006). Whilst both reduce consumption, on this analysis only true aversion would change the hedonic value of the flavor thus reducing palatability, while avoidance would describe the situation where consumption changes without the development of aversive hedonic reactions. Consistent with this analysis, it has been demonstrated that – despite equivalent effects on overall intake – pairing a taste with nausea induced by LiCl in rats subsequently results in aversive responses to the taste, while pairing the taste with pain induced by an intraperitoneal injection of hypertonic NaCl, which produces pain but not nausea, results in the taste eliciting immobility reflecting fear (Dwyer et al., 2017). Therefore, it has been suggested that in order to produce an increase in aversive hedonic reactions, CTA requires the flavor to be associated with visceral illness that induces nausea and vomiting in emetic species (Garcia et al., 1968; Pelchat et al., 1983). Although there are alternative theoretical positions that interpret such changes in the learned hedonic responses to flavors in terms of quantitative differences instead of qualitatively different types of learning (Lin, Arthurs, et al., 2012; Lin et al., 2014), the idea that there are informative dissociations between overall consumption and responses indicative of hedonic reactions remains. While the current study does not seek to directly compare CTA to AN, this example from CTA displaying (partial) dissociations between consumption and affective measures illustrates the potential for applying affective measures to AN to illuminate the mechanisms underpinning AN.

Two common methods applied to assess palatability are the taste reactivity test (TRT) (Grill & Norgren, 1978) and analysis of licking microstructure patterns (Davis & Levine, 1977; Davis & Smith, 1992; Dwyer, 2012). The TRT involves assessing orofacial patterns reflecting affective reactions to a flavored solution. The orofacial responses are classified as appetitive, aversive or neutral. In this procedure the solutions are usually infused via a surgically implanted intraoral cannula (Berridge, 2000; Dwyer, 2012; Neath et al., 2010). In this way, TRT provides a measure of taste palatability while reducing approach and preparatory behaviors to a minimum, thus focusing on the hedonic qualities instead. In contrast, the analysis of licking microstructure examines the pattern of ingestive behavior through recording timing of licks during voluntary solution consumption. Rodents drink in rapid rhythmic licking blocks called clusters, which last for a variable length of time depending on the palatability of the flavor (Ahmed et al., 2017; Dwyer, 2012). The key hedonic measure is the mean size of lick clusters (Lick Cluster Size – in this paper, the acronym LCS will be used both to indicate the measurement parameter itself, and as a term for the analysis procedure more generally): LCS is greater if the flavor is liked (Davis & Smith, 1992; Lin, Amodeo, et al., 2012), while LCS will be lower if the flavor is disliked (Lin, Amodeo, et al., 2012). In this test, the animal voluntarily approaches the bottle and therefore both consumption and taste palatability data are obtained.

Returning to the application of the TRT and LCS techniques, one interesting class of observation comes from dissociations between these affective measures and measures of consumption. For example, following the induction of a CTA, both the reduced consumption and palatability attenuate dramatically with exposure to the flavor alone, regardless of the method used to assess palatability (Dwyer, 2012; Lin et al., 2014; Parker, 2003). However, consumption recovers more slowly than palatability measured either with TRT (Cantora et al., 2006) or LCS (Dwyer, 2009), and while the hedonic value of the flavor typically returns to pre-conditioning levels, consumption does not always reach that level (Rosas & Bouton, 1996). That is, a persistent reduction in the palatability of the target flavor following CTA does not seem to be responsible for the persistent suppression of consumption. It is not yet possible to make a similar statement about AN, because while small number of AN studies have also applied these affective measures, different results have been obtained depending on the method applied to assess the palatability shift. Lin, Amodeo, et al. (2012) reported increased LCS after four exposures to saccharin or quinine solutions. In contrast, Neath et al. (2010) found no increase in appetitive reactions using TRT after five consecutive exposures to different concentrations of saccharin, even though a subsequent consumption test indicated that AN had taken place. This latter result was interpreted as suggesting AN does not increase taste palatability, but rather the tendency to approach the bottle (and in turn, suggesting AN involves a change in wanting but not liking). Thus, depending on whether TRT or LCS methods are used, there is evidence both for and against the idea that AN involves a change in flavor palatability – creating an ambiguity that could be resolved by re-examining AN using both TRT and LCS methods.

Up to this point, we have discussed the phenomena of AN in general, but it is important to consider whether the presence or degree of the effect is indeed universal. While no effects of sex have typically been reported in AN assessed using only intake measures with either cider vinegar (3 %) or sodium saccharin (.1 %) solutions (Expósito et al., 2023), there are, to our knowledge, no data available using TRT or LCS in AN experiments comparing male and female animals. In fact, most previous experiments were performed only in male rats. Nevertheless, sex differences in taste reactivity have been found using TRT (Clarke & Ossenkopp, 1998; Flynn et al., 1993) and licking rate (Curtis et al., 2004). Female rats exhibit more appetitive reactions than male rats in response to sucrose (.3 M), quinine (.0003 M) (Clarke & Ossenkopp, 1998) and low NaCl concentration (Flynn et al., 1993) solutions. Females also lick at higher rates than males for NaCl and sucrose solutions, and these sex differences do not seem to be fully explained by hormonal differences. This is, studies on ovariectomized female rats found that these sexual differences in licking persisted regardless of manipulations of sex hormones (Curtis et al., 2004). Thus, the present studies will compare male and female rats.

In addition, genetic influences in overall hedonic responses have been identified. For example, *CACNA1C* is a gene that encodes Cav 1.2 L-type voltage-gated calcium channels (which are expressed in brain, heart, and other tissue), and has been strongly associated with multiple psychiatric disorders (including bipolar, schizophrenia, depression) where affective symptoms are commonly reported (Ferreira et al., 2008; Mullins et al., 2021; Ripke et al., 2014; Trubetskoy et al., 2022). A rodent model of *CACNA1C* hypofunction is the *cacna1c*$^{+/-}$ heterozygous rat, which is a constitutive zinc finger nuclease knockout, resulting in an approximately 50 % and 40 % decrease in hippocampal mRNA and

protein levels, respectively (Moon et al., 2018; Sykes et al., 2019). The *Cacna1c*$^{+/-}$ rat has recently been shown to exhibit reduced appetitive reactions to palatable solutions (Gasalla et al., 2025). This is consistent with the reported altered expression of *CACNA1C* associated with anhedonia in psychiatric patients (Dedic et al., 2018). However, it is not known whether this genetic alteration of hedonic reactions extends to solutions other than palatable sucrose or whether it impacts AN. Thus Experiment 1 examined AN using both *cacna1c*$^{+/-}$ rats and wild-type controls (but to prefigure the results, no differences between *cacna1c*$^{+/-}$ rats and wild-type controls were observed in Experiment 1, and so *cacna1c*$^{+/-}$ rats were not used in Experiment 2).

In order to explore the nature of palatability changes induced by AN in rats, we designed two experiments, Experiment 1 applying LCS and Experiment 2 using TRT, with repeated exposures to cider vinegar. This flavor has been extensively used in our laboratory, and has demonstrated the induction of a clear neophobia response and a progressive attenuation (Expósito et al., 2020, 2023; Grau-Perales, Gómez-Chacón et al., 2019). Moreover, vinegar is a mildly unpleasant taste for rats (in contrast to the saccharine used by Neath et al. (2010) in their prior investigation of AN using TRT methods), and so using this allows for a more direct comparison between TRT and LCS methods than was possible in previous studies. In both experiments consumption was also recorded either during the procedure (Experiment 1) or at the end (Experiment 2) by adding an additional voluntary intake test. We hypothesize generally that if the palatability of a novel taste increases as it is encoded as safe, it will be reflected in progressively higher consumption, LCS and increased appetitive reactions, while aversive/-passive reactions will decrease Finally, given that previous data have been obtained in males and there are sex-dependent differences in flavor processing, we included both sexes in order to explore potential sex-dependent differences in the relationship between AN and hedonic reactions.

2. Method

2.1. Experiment 1 – LCS

2.1.1. Subjects

Forty-two adult male and female *cacna1c* heterozygous (*cacna1c*$^{+/-}$, HET) on a Sprague-Dawley background (TGR16930, Horizon, Sage Research Labs, USA) and wild type (WT) littermates were obtained and housed in mixed-genotype, single sex groups of 2–3 in standard cages (38 × 56 × 22 cm) per cage. Of these, there were 27 males (450 ± 7.32 g) and 15 females (258 ± 4.94 g), distributed in 4 groups: MaleHET (n = 9), MaleWT (n = 18), FemaleHET (n = 5) and FemaleWT (n = 10). Rats were bred and housed at Cardiff University (United Kingdom) under a 12 h/12 h light/dark cycle with all manipulations taking place in the light cycle. Animals had ad libitum water and food until the experiment started and a water restriction schedule imposed and were handled daily for a week prior to the start of experimental procedures and during the acclimatization to water restriction. This water restriction remained until the end of the experiment. Experiments were conducted in accordance with local ethic guidelines, the UK Animals (Scientific Procedures) Act 1986 (project license number PP3468526).

2.1.2. Stimulus and apparatus

Fig. 1A shows a schematic illustration of the LCS equipment. The rats were tested for both water and vinegar sessions in 15 drinking chambers (Med Associated Inc, United States). These chambers measured 32 × 15 × 12 cm (L × W × H), with steel mesh flooring and white acrylic walls. The drinking chambers were located in a room separate from the home cages. Fluids were accessible through drinking spouts made of stainless steel, attached to 50-ml cylindrical bottles. These could be inserted on the left- or right-hand side of the lid (made of wire mesh with a plastic spout holder). Spouts were placed level with the front of the spout holder (to minimize non-drinking contacts) and fluid bridges were minimized by the plastic spout holder insulating from the wire mesh of the lid, with the spout holder dried between runs. The distance between the holes for the bottles was 8 cm. Only the left-hand side was used for the present experiment. A contact-sensitive lickometer registered the time of each lick to the nearest .01 s. This was recorded by a computer using MED- PC software (Med Associates, Inc., St. Albans, VT). The amount of fluid consumed by each rat was measured by weighing the drinking bottle before and after each session. The stimuli were tap water or a solution of 3 % (w/w) cider vinegar (Vin).

2.1.3. Procedure

These procedures were carried out in the Behavioural Neurosciences Laboratory (BNL) of the School of Psychology of Cardiff University. The animals were water deprived in the afternoon of the day before the beginning of the experiment. The experimental sessions took place in the morning (at 11:00 a.m.). There was an initial period of 5 daily sessions of 15-min access to water, in order to habituate rats to the licking boxes (the last of these comprised the water baseline day). Subsequently, there were 6 daily 15min sessions of access to 3 % vinegar solution (Vin days) in the drinking chambers, while the licking patterns were being recorded. Each day, animals were weighed and had a rehydration session in the afternoon (at 3:00 p.m.), which consisted of a 1 h session of free-access to tap water in their home cage. Although home cage water consumption was not measured, animals had typically finished drinking well before the completion of the 1-hr water access period, suggesting they were fully replete by the end of this period. Thus, animals were tested with 19hrs between the end of rehydration session and the start of the experimental session. Other than transient weight loss following the initial imposition of the water restriction schedule, no detrimental weight changes were observed.

2.1.4. Data analysis

Due to technical problems disrupting lick recording, the fifth exposure to cider vinegar solution (Vin5) was excluded from the analyses. In addition to the intake data, the mean LCS for each rat was extracted from the record of licks for analysis. A cluster was defined as a set of licks, each separated by an inter-lick interval of no more than .5 s. This criteria has been used in the bulk of lick analysis work performed in the Cardiff lab (Dwyer, 2009; Dwyer et al., 2013; for a review, see Dwyer, 2012). Mixed Linear Models (MLM) was used to analyse the test data with the two between-group factors Genotype (HET vs. WT), and Sex (male vs. female) plus Day as a within-subject factor (Baseline, Vin 1, Vin2, Vin3, Vin4, Vin6). In addition, the total number of lick clusters and the mean inter-lick interval (ILI) within licking clusters were examined. The ILI is a measure of lick rate, and is primarily used in our lab as an indicator of lick recording problems because licking rate is typically constrained within tight bounds (see below for exclusion criteria). Independent MLM analyses 2 (Genotype) × 2 (Sex) × 6 (Day) were carried out for each variable. All data were analyzed using SPSS (IBM, United States). All tests reported here used a criterion for significance of $p < .05$. LSD post-hoc tests based on the marginal means were used to explore the differences yielded by the analyses.

MLM was used rather than ANOVA in order to deal with the fact that there were a number of missing datapoints. These missing points related to unsystematic equipment failures (e.g. bottle blockages or leaks, lick recording failures). For this purpose, three variables were used to identify datapoints where the lick analysis would be compromised and were thus excluded: intake, volume/1000 licks and ILI. For intake, data for days where consumption was less than 0.5 g were excluded (Davis & Smith, 1992; Dwyer, 2009; Riordan & Dwyer, 2019). For volume/1000 licks, exclusions were made for values less than 3 g or greater than 10 g. While for ILI, exclusions were made for values less than 130ms or larger than 160ms (Dwyer et al., 2008; Riordan & Dwyer, 2019).

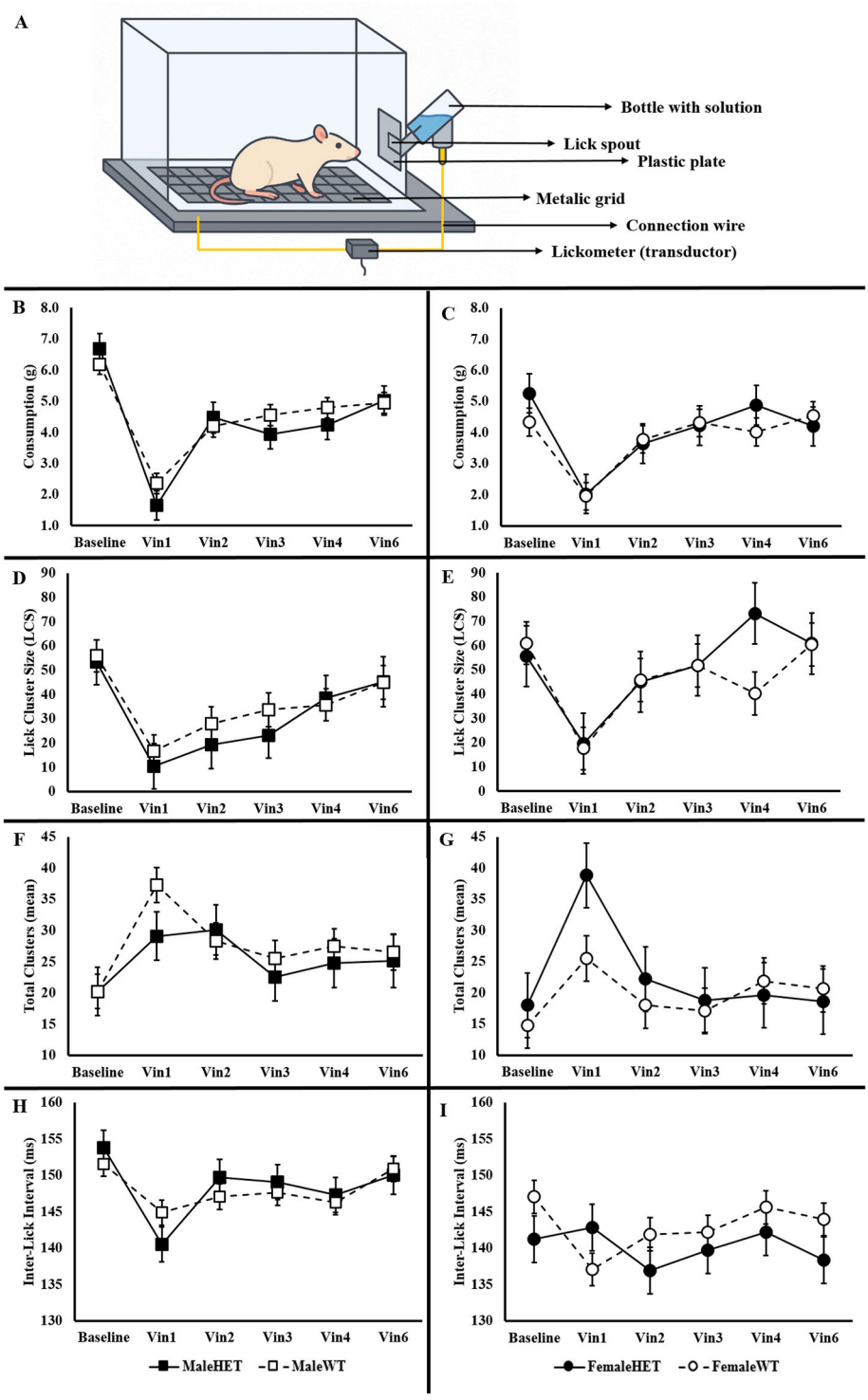

Fig. 1. Microanalysis of licking structure patterns procedure and results. Fig. 1 A shows a schematic representation of the licking box setup: the rat is placed into a plastic box on a metal grid that is part of an open electrical circuit, and each lick on the bottle spout closes the circuit, enabling the device to register lick-related parameters such as frequency and latency. Fig. 1 B and C shows consumption from males (1 B) and females (1 C): effects of Day and Sex × Day, indicating that males' vinegar consumption never reached baseline water levels, while females did. Fig. 1 D and E shows Lick Cluster Size from males (1 D) and females (1 E): effects of Day and Sex, due to females had an average LCS higher than males. Fig. 1 F and G shows total clusters from males (1 F) and females (1 G): main effect of Day was found. Fig. 1 H and I shows the average inter-lick interval of males (1 H) and females (1 I): effects of Day, Sex, Sex × Day, Genotype × Sex × Day, but these do no suggest an impairment in consumption behaviour. Wild Type group is represented with black and continuous lines. Cacna1C HET group is represented with white and dashed line. Data is shown as mean and error bars show +/− × 1SEM. Panels for different measures use different scales.

2.2. Experiment 2 – TRT and voluntary intake test

2.2.1. Subjects

Forty-seven adult Sprague-Dawley rats (ENVIGO, Netherlands) (Males: n = 23, 328 ± 1.93 g; Females: n = 24, 218 ± 1.67 g) were used. They were housed in standard home cages (38 × 56 × 22 cm) in groups of 3–4 animals per cage. After surgery, all animals were randomly assigned to a group depending on the solution infused during TRT either a 3 % cider vinegar solution (Experimental group) or tap water (Control group). There were a total of 4 groups: MaleExp (n = 12), MaleControl (n = 11), FemaleExp (n = 12) and FemaleControl (n = 12). Rats were housed in the animal facilities located in the Biomedical Research Center (CIBM) of the University of Granada (Spain) under a 12 h/12 h light/dark cycle, taking place all manipulations during the light cycle. They had ad libitum water and food until the experiment started and received 3 min daily handling sessions for the previous 4 days to the surgery. The procedures were approved by the University of Granada Ethics Committee for Animal Research and by the Regional Ministry of Agriculture, Fisheries and Rural Development of Andalusia (1/06/2022/078).

2.2.2. Surgery

Animals were surgically implanted with an intraoral cannula. Surgery was carried out following the method described on Parker (1995) and widely used in the Cardiff lab (Dwyer et al., 2017; Gasalla et al., 2016, 2017; López et al., 2022). First, they were anesthetized using ketamine (50 mg/kg, i.p.) combined with medetomidine clorhydrate (.15 mg/kg, i.p.). In order to implant the cannula a thin-walled 14-gauge stainless steel needle was inserted at the back of the neck, directly subcutaneously around the ear and routed into the oral cavity, exiting behind the first molar. A length of polyethylene tubing with an inner diameter of .86 mm and an outer diameter of 1.27 mm was then run through the needle after which the needle was removed. The tubing was secured with a rubber disc placed over and drawn to the exposed skin at the back of the neck, to stabilize the cannula. The tubing was held secure in the oral cavity by an O-ring and a rubber stopper, which were sealed behind the tubing prior the cannulation surgery. Finally, the animal received subcutaneous injections of atipamezol (2.5 mg/kg; an anaesthesia-reversing drug), buprenorphine (.075 mg/kg; anti-inflammatory drug), enrofloxacine (12.5 mg/kg; antibiotic drug) and 5 % glucose (5 ml for males and 4 ml for females; nutrient replacement). The animals were returned to an individual home-cage (43 × 27 × 19 cm) and the solid food was removed until the next day. They were allowed to recover for 3 further days, receiving analgesic and antibiotic subcutaneous injections each day and the cannulae flushed with tap water each day.

2.2.3. Equipment

Fig. 2A shows a schematic illustration of the TRT equipment. The test was carried out in a dark room, lit with two manipulable spotlights that allowed control of the light conditions for recording. Two transparent methacrylate boxes equipment were used simultaneously for video recording TRT. Each of these was formed by a methacrylate holder structure (28 × 24 × 55 cm) and a methacrylate box (27 × 24 × 21 cm) with a pierced removable lid. A webcam plugged to a laptop was under the box, recording it from the bottom. After each trial, each box was cleaned using an odorized surface cleaner (Sanytol Multiusos Eucalyptus, Barcelona, Spain) and was allowed to ventilate for few minutes in order to neutralize any vinegar odor prior to the next subject. Animals were assigned to boxes counterbalanced across group and sex to control possible extraneous variables. 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 a model 55–2226 infusion pump (Harvard Apparatus, United States) (for a detailed description of the equipment and procedure, see López et al., 2022).

2.2.4. Procedure and criteria for TRT analysis

The procedures were carried out in the Animal Behavior Analysis Unit of the Biomedical Research Center (CIBM) of the University of Granada. Three days after surgery, and upon full recovery of animals from surgery, testing began. Table 1 describes the behavioral procedure applied. On Day 1 (Baseline day) habituation to the TRT chamber and infusion procedure took place. All groups received 1 ml/min intraoral infusion of water for 5 min (Pre-Water). From Day 2 to Day 7, the rats were infused at the same rate (1 ml/min, for 5 min) with 3 % cider vinegar solution or water depending on group assignment. On Day 8, all groups received a water session again (Post-water). Until Day 8, all animals had free-access to water in their home cage. On the afternoon of Day 8 animals were water-deprived to prepare them for voluntary access to water during the subsequent daily 15 min morning sessions held at 11:00 a.m. From Day 9 to Day 11, tap water was available in pre-weighed bottles that were weighed again after the session. After establishing the baseline for three days in which water was available during the experimental sessions, animals had access to a 3 % cider vinegar solution instead of water for the following three days (Days 12–14). Each afternoon at 4:00 p.m., they were provided with free access to water for 30 min until they were replete, in a similar way as was done in Experiment 1.

The criteria for TRT analysis used in this experiment are described in detail in López et al. (2022), which in turn reflects the initial development of the TRT by Grill and Norgren (1978). In brief, orofacial reactions are classified as appetitive, aversive and neutral. Appetitive: tongue protrusions, mouth movements and paw licking. Aversive: gaping, chin-rubbing, paw treading, forelimb flicks and head shakes. Neutral: passive dripping. The appetitive reaction score is the total duration (in seconds) spent displaying these reactions. The aversive reaction score is the sum of times that these reactions were displayed.

2.2.5. Data analysis

TRT videos were analyzed using BORIS software (Friard & Gamba, 2016). Behaviors were coded and a researcher was assessing each video individually. A researcher blind to the experimental procedures analyzed some of the videos to confirm that the criteria used was accurate. Mixed analyses of variance (ANOVAs) were applied to the results obtained with TRT and consumption with the between-group factors Group (Vin vs. Water) and Sex (Male vs. Female) and Day as within-subject factor. This resulted in three 2 (Group) × 2 (Sex) × 8 (Day) mixed ANOVAs for analyzing each TRT behavioral category (aversive, appetitive, and passive dripping) and a 2 (Group) × 2 (Sex) × 6 (Day) mixed ANOVA for analysing consumption. All analyses were carried out using SPSS (IBM, United States). All tests reported used a criterion of significance of $p < .05$. When assumption of sphericity was violated (Mauchly's sphericity test: $p < .05$), Greenhouse-Geisser correction was used. LSD post-hoc tests based on the marginal means were used to explore the differences yielded by the mixed ANOVAs.

3. Results

3.1. Experiment 1

Fig. 1 shows the results obtained in Experiment 1. LSD post-hoc tests were used to explore the differences yielded by the MLM.

Fig. 1B and C displays the results for Consumption. A 2 (Genotype) × 2 (Sex) × 6 (Day) MLM analysis revealed a significant effect of Day [$F(5, 184.772) = 44.388$, $p < .001$] and Sex × Day interaction [$F(5, 184.772) = 3.113$, $p = .010$] but not Genotype × Sex [$F(1, 37.443) = .287$, $p = .595$], Genotype × Day [$F(5, 184.772) = 1.182$, $p = .320$] or Genotype × Sex × Day [$F(5, 184.772) = .953$, $p = .448$] interactions. Pairwise comparisons of the estimated marginal means were carried up as follow-up tests of the main effects. Analysis of the Day effect revealed that water baseline intake was higher than any other individual day (Baseline vs. each Vin day: $ps < .001$), which means that cider vinegar consumption

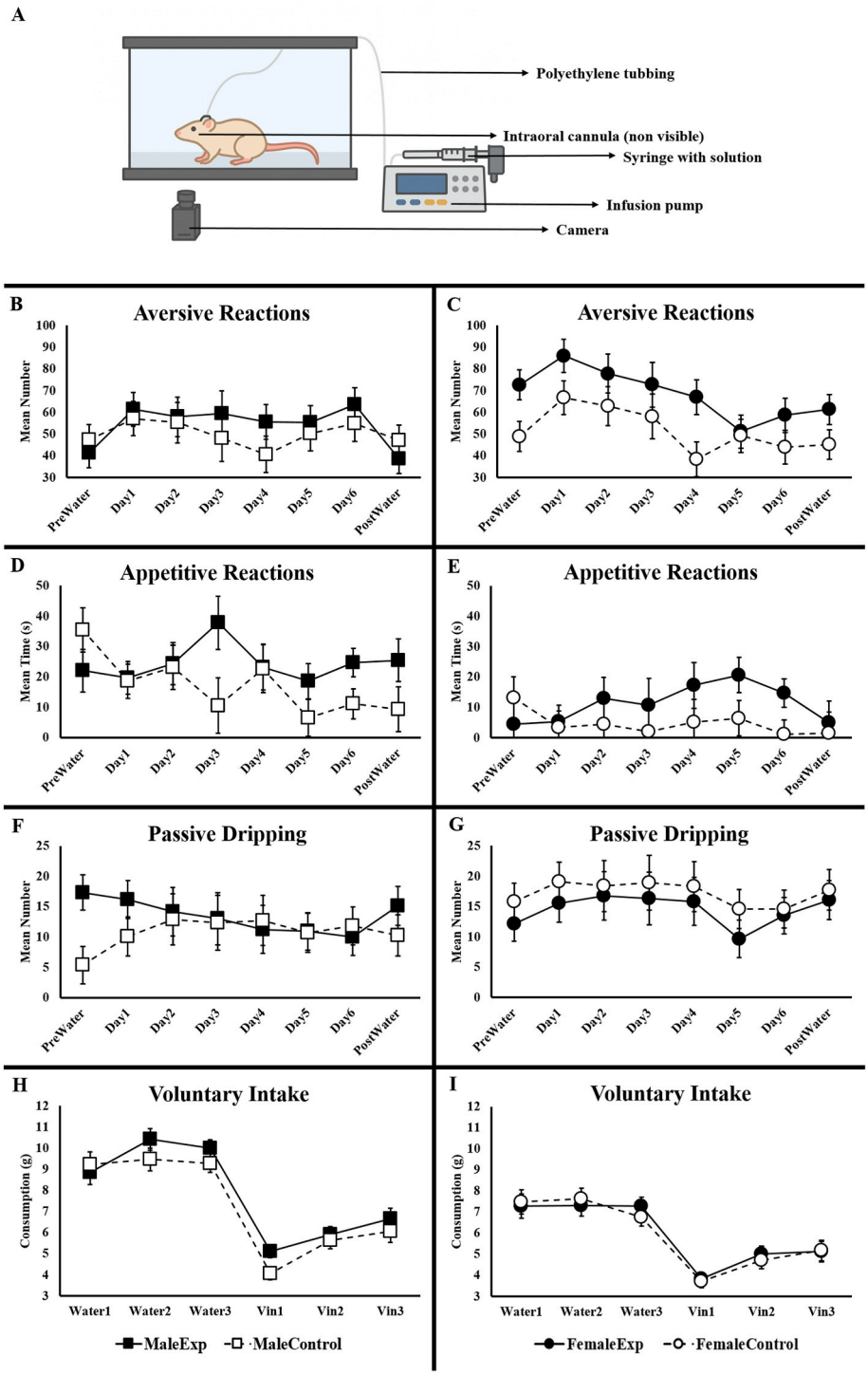

Fig. 2. Taste reactivity test procedure and results. Fig. 2 A shows a schematic representation of the taste reactivity test setup: an infusion pump delivers the flavored solution at a constant rate through an intraoral cannula directly into the oral cavity of the rat, and orofacial reactions are recorded by a camera positioned beneath the animal. Fig. 2 B and C shows mean number of aversive reactions of males (2 B) and females (2 C): effects of Day and Sex × Day indicates that females displayed a higher number of aversive reactions than males on PreWater and Vin1 days. Fig. 2 D and E shows mean seconds displaying appetitive reactions of males (2 D) and females (2 E): there were main effects of Day, Group (Vin exposed group displayed more appetitive reactions than Control group) and Sex (males showed more appetitive reactions than females). Fig. 2 F and G shows the mean passive dripping of males (2 F) and females (2 G): no effects were found. Fig. 2 H and I shows mean consumption of males (2 H) and females (2 I) during the baseline (Water1-3) and experimental (Vin1-Vin3) sessions during Voluntary Intake test for each group: effects of Day, Sex and Sex × Day, due to average consumption of males was higher than of females, and, specifically, this happened mainly in the baseline days. Experimental groups are represented with black and continuous line. Control groups are represented with white and dashed line. Data is shown as mean and error bars show +/− × 1SEM. Panels for different measures use different scales.

Table 1
Experimental design of Experiment 2.

Group	D1 TRT	D2 TRT	D3 TRT	D4 TRT	D5 TRT	D6 TRT	D7 TRT	D8 TRT	D9-D11 Water Deprivation	D12 VI	D13 VI	D14 VI
Exp	Wat	Vin1	Vin2	Vin3	Vin4	Vin5	Vin6	Wat	Wat	Vin7	Vin8	Vin9
Control	Wat	Wat	Wat	Wat	Wat	Wat	Wat	Wat	Wat	Vin1	Vin2	Vin3

Exp = Experimental; D = Day; TRT = Taste Reactivity Test; VI = Voluntary Intake; Wat = Water; Vin = Vinegar.

never reached the previous water consumption levels when averaging over male and female animals. In addition, Vin1 consumption was also significantly lower than any other individual Vin day, indicating attenuation of neophobia from Vin2 day onwards (Vin1 vs. each other Vin day: $ps < .001$). However, there were no differences in consumption between Vin2 and Vin3 ($p = .362$), but consumption increased gradually during the following exposures, with significant differences between Vin2 and Vin6 ($p = .012$). There were no differences in consumption between Vin3, Vin4 or Vin6 ($ps > .05$), suggesting neophobia was attenuated at the third exposure. However, the Sex × Day interaction indicated a different attenuation pattern between males and females. While flavor neophobia in females was completely attenuated on Vin3, since the consumption of vinegar solution did not differ with baseline water (Baseline vs. Vin3: $p = .193$) and the subsequent days (Baseline vs. Vin4: $p = .394$; Baseline vs. Vin5: $p = .299$), this was not the case of males, which never consumed as much vinegar solution as they did water (Baseline vs. Vin6: $p < .001$), although the vinegar consumption did stabilized gradually with no significant differences between Vin5 and Vin6 consumption (Vin5 vs. Vin6: $p = .128$). The comparison between the consumption of males and females in each day yielded significant differences only on Baseline, as intake of males was higher than females ($p = .001$), but did not differ on each individual Vin day (lowest p value for the male vs. female comparison for Vin days was $p = .209$).

LCS results are shown in Fig. 1D and E. The 2 (Genotype) × 2 (Sex) × 6 (Day) MLM showed a significant effect of Day [$F(5, 181.282) = 13.339, p < .001$] and Sex [$F(1, 38.223) = 5.700, p = .022$]. No effects of Genotype [$F(1, 38.223) = .002$, $p = .963$], Genotype × Sex [$F(1, 38.223) = .532$, $p = .470$] or the interactions Genotype × Day [$F(5, 181.282) = 1.222$, $p = .300$], Sex × Day [$F(5, 181.282) = 1.131$, $p = .345$] or Genotype × Sex × Day [$F(5, 181.282) = .517$, $p = .763$] were found. Females displayed higher LCS than males presumably indicating increased palatability of the cider vinegar solution, although some other factor cannot be excluded. Analysis of the Day effect revealed higher LCS in the baseline day than on Vin1 ($p < .001$) and lower LCS on Vin1 day than in any other of each subsequent vinegar day (the higher p-value of Vin1 vs. any other Vin day was $p = .002$), consistent with reduced palatability to the novel solution. Baseline LCS were also higher than Vin2 ($p < .001$) and Vin3 ($p = .004$), but there were no differences with Vin4 ($p < .094$) or Vin6 ($p < .537$). This indicated a reduced palatability to a neophobic vinegar solution which recovered completely reaching the level of water baseline when the flavor becomes familiar and safe after several exposures. The process involved a progressive increase in LCS over the repeated exposures with respect to Vin1. In fact, animals displayed a significantly higher LCS on Vin2 than on Vin1, but Vin2 had no differences with Vin3 ($p = .332$). Nevertheless, Vin2 still being significantly lower than Vin4 ($p = .031$) and Vin6 ($p = .002$). Vin3 was not significantly different to Vin4 ($p = .227$), but LCS on Vin3 was lower than on Vin6 ($p = .028$). There were no differences between Vin4 and Vin6 ($p = .305$). Hence, this process parallels that of consumption albeit somewhat slower to reach terminal levels.

Total number of clusters results are shown in Fig. 1F and G. The 2 (Genotype) × 2 (Sex) × 6 (Day) MLM showed a significant effect of Day [$F(5, 180.416) = 9.427, p < .001$] and a marginally significant effect of Sex [$F(1, 37.534) = 3.710$, $p = .062$]. No other main effect or interactions were significant: Genotype [$F(1, 37.534) = .020$, $p = .887$], Genotype × Sex [$F(1, 37.534) = .918$, $p = .344$], Genotype × Day [$F(5, 180.416) = .519$, $p = .762$], Sex × Day [$F(5, 180.416) = .709$, $p = .617$] or Genotype × Sex × Day [$F(5, 180.416) = 1.637$, $p = .152$]. Males produced non-significantly more clusters than females, which is the converse of the LCS results (that is, overall, females produced similar numbers of licks to males but in fewer clusters - reflecting the overall higher LCS in females). Across days, there was an initial increase in the number of clusters on Vin1 (reflecting the fact that although consumption was lowest on this day, the LCS was also lowest - so a small number of licks was distributed among a larger number of clusters), and the number of clusters decreased across Vin days (reflecting the recovery in LCS, so the increasing consumption and total licks were distributed among fewer clusters). Although this Vin1 increase in the number of clusters was numerically highest in Female HETs, there was no significant Genotype × Day × Sex interaction.

Finally, average ILI is shown in Fig. 1H and I. The 2 (Genotype) × 2 (Sex) × 6 (Day) MLM showed several significant effects: Day [$F(5, 179.833) = 6.979, p < .001$], Sex [$F(1, 37.231) = 12.788, p < .001$], Sex × Day [$F(5, 179.833) = 2.890, p < .016$], Genotype × Sex × Day [$F(5, 179.833) = 3.587, p < .004$]. No other significant main effects of Genotype [$F(1, 37.231) = 12.788, p = .518$], Genotype × Sex [$F(1, 37.231) = .702, p = .407$] or Genotype × Day [$F(5, 179.833) = .519, p = .762$] were found. The shorter ILI (more rapid intra-bout lick rate) in females as opposed to males reflects their generally smaller size. The effect of Day reflected an initial lowering of ILI on first exposure to Vin, which recovered across sessions. The Sex × Day interaction reflects the fact that the difference across days was more pronounced in males than females; and in turn the Genotype × Sex × Day interaction reflects the fact that the male/female difference in the size of the Day effect was most pronounced for HET as opposed to WT animals. However, the main concern with ILI is that materially raised ILIs (e.g. reflecting impaired motor behavior) can artificially suppress LCS - but the current results indicate only minor differences in ILI, and these are the opposite of the pattern expected to reflect a motor impairment (i.e. ILIs were lowest at the point where LCS was lowest).

In summary, Experiment 1 demonstrated the typical consumption patterns with AN, a suppression of consumption of vinegar below water baseline levels on first exposure followed by a progressive increase (which reached baseline levels in females but not males). This was accompanied by initial low hedonic responses to vinegar as indicated by low lick cluster size, and this also recovered (albeit more gradually) over exposures. There were no differences between cacna1c HET and WT animals, while female rats generally consumed less than males, but with generally higher lick cluster sizes.

3.2. Experiment 2

Fig. 2 shows the results obtained in Experiment 2. Concerning the TRT results, Fig. 2B and C presents the mean number of aversive reactions of each group in the daily 5-min TRT session for Males and Females, respectively. A mixed 2 (Group) × 2 (Sex) × 8 (Day) ANOVA yielded significant effects of Day [$F(7, 301) = 5.136, p < .001$] and Sex × Day [$F(7,301) = 2.216, p = .033$], but not Group [$F(1, 43) = 3.200, p = .081$], Sex [$F(1, 43) = 1.842$, $p = .182$], Group × Sex [$F(1, 43) = 1.174, p = .285$], Group × Day [$F(7, 301) = .904, p = .503$] or Group × Sex × Day interaction [$F(7, 301) = .785$, $p = .600$]. In order to explore the Sex × Day interaction, a mixed 2 (Group) × 8 (Day) ANOVA for each sex was carried out. This confirmed a significant Day effect ($p < .001$) in females but not an effect of Group [$F(1, 22) = 3.966, p = .059$] or Group × Day interaction [$F(4.025, 88.553) = .882$, $p = .479$]. In contrast, males did not show any significant effect (the smallest p-value was $p >$

.053) Focusing on the pattern of differences across days, females displayed a significant higher number of aversive reactions than males on PreWater ($p = .023$) and on Vin1 ($p = .032$). However, is important to highlight that, since there were no differences in the pattern of aversive reactions between groups receiving water and vinegar, there is little evidence of neophobia or attenuation attending the hedonic changes using TRT.

Fig. 2D and E shows the mean number of appetitive reactions. A mixed 2 (Group) × 2 (Sex) × 8 (Day) ANOVA was carried out. There were significant effects of Group [$F(1, 43) = 4.149$, $p = .048$] as the group exposed to the vinegar solution displayed higher number of appetitive reactions than the group exposed to water; and Sex [$F(1, 43) = 13.747$, $p = .001$], where males displayed a higher number of appetitive reactions than females. No other effects were significant.

Fig. 2F and G presents the mean number of neutral passive dripping reactions of each group. The 2 (Group) × 2 (Sex) × 8 (Day) mixed ANOVA did not yield any significant effects. In this regard, passive dripping appears to be insensitive to any of the manipulations carried out.

Finally, Fig. 2H and I shows the consumption of each group during voluntary intake test carried out after the TRT sessions including 3 water days and 3 vinegar exposures. A 2 (Group) × 2 (Sex) × 6 (Day) mixed ANOVA yielded significant effects of Day [$F(5, 215) = 88.956$, $p < .001$], Sex [$F(1, 43) = 33.971$, $p < .001$] and the interaction Sex × Day [$F(5, 215) = 3.753$, $p = .003$]. There were no significant effects of Group [$F(1, 43) = 1.172$, $p = .285$], Group × Day [$F(5, 215) = .638$, $p = .671$], Group × Sex [$F(1, 43) = .700$, $p = .407$] or Group × Sex × Day [$F(5, 215) = .492$, $p = .782$]. The Sex effect was due to the fact that males had an average consumption higher than females. The analysis of the Sex × Day interaction yielded a Day effect for both males ($p < .001$) and females ($p < .001$). For males, Water3 consumption was significantly higher than any of the Vinegar days (Water3 vs. any individual Vin day: $p < .001$) reflecting flavor neophobia. On Vin2, consumption was significantly higher than on Vin1 ($p = .002$), indicating AN, whilst there were no differences between Vin2 and Vin3 ($p = .159$). In the case of the females, Water3 consumption was higher than on all Vinegar days (Water3 vs. each individual Vin day: $p < .001$), and Vin1 had a significant lower consumption than the following days (Vin1 vs. each of Vin2 and Vin3: $p < .001$), so there was flavor neophobia; a greater consumption on Vin2 reflects AN, whilst there were no differences with Vin3 ($p = .321$). Comparing males and females, males had a significant higher consumption on all days ($ps < .022$) although the differences were higher for water than vinegar.

In summary, in Experiment 2 the TRT was not sensitive to the difference between vinegar and water (although there were TRT differences between males and females) and nor did intra-oral exposure to vinegar during the TRT sessions result in any differences in later voluntary consumption tests.

4. Discussion

Experiment 1 indicated the typical pattern of AN with consumption of vinegar low on first exposure and increasing across days (although this only reached the level of water consumption in females and not males). In addition, the microstructure of licking analysis revealed an initial suppression of LCS on first exposure to vinegar that progressively increased across days (albeit over a different timescale to the increase in consumption). There were further sex differences with consumption being generally higher in males than females, but LCS generally higher in females. There was no effect of the *cacna1c* manipulation. In Experiment 2, the TRT hedonic measure did not reveal any overall differences between vinegar and water, nor a systematic change in hedonic reactions over successive intra-oral exposures to vinegar. In addition, subsequent voluntary consumption tests showed the normal pattern of AN, but no effect of prior intra-oral exposure to vinegar. There were again sex differences in the overall levels of consumption and hedonic reactions. These results raise a number of issues that we will consider in turn.

Concerning palatability and AN, the results obtained using LCS are consistent with previous reports using different tastes. In line with the results of Lin, Amodeo, et al. (2012) who reported increased LCS in male rats after four saccharin and quinine exposures, we found reduced LCS in response to the novel vinegar solution which increases after four exposures reaching the asymptote both in males and females. Thus, our results extend those of Lin, Amodeo, et al. (2012) to acid tastes and both sexes. However, even though AN shares with CTA extinction a pattern of increased consumption across flavor-alone exposures, the results of Experiment 1 do not seem to parallel those obtained during extinction of CTA. In Experiment 1 consumption recovered abruptly and reached the asymptote on the third day. LCS, however, increased gradually across days. This pattern is different to that described during CTA extinction. Dwyer (2009) reported a gradual increase of consumption never reaching the asymptote, while LCS changes were faster and larger (Dwyer, 2009; Dwyer et al., 2013). Although relying on cross-experiment comparisons, the difference in the relative patterns of LCS and consumption changes between AN and CTA extinction may suggest different underlying learning mechanisms. It is conceivable that CTA extinction involves learning a previously unsafe flavor is now safe, while AN would involve learning an unknown flavor was safe. The first would require overcoming previous aversive experiences while the second only would require assessing the effect of a non-familiar flavor. In the context of CTA, it has been suggested that preparatory avoidance responses may inhibit consumption but have little effect on hedonic reactions, which could explain the faster extinction of conditioned palatability reactions in CTA (Parker, 2003). In contrast AN does not involve overcoming learned avoidance, potentially allowing intake to recover swiftly. Even if some flavors have inherent properties that make them, at least initially, unpreferred (e.g., fermentative acidity could be related with harmful microorganisms), it would seem maladaptive for a water-restricted animal to reject flavors that have no negative consequences after ingestion.

In contrast, Experiment 2 did not find any TRT changes selectively associated with neophobia or AN, meaning our hypotheses that both appetitive and aversive TRT reactions would be sensitive to neophobia and its attenuation must be rejected. Indeed, taken at face value the fact that vinegar triggered a higher number of responses than water suggests that flavor exposure, even when it is “mildly-aversive”, is more appetitive than the exposure to a non-flavored solution. Experiment 2 also indicated that the intraoral infusion did not reduce flavor neophobia: surprisingly, the Experimental group previously exposed six times to the vinegar solution during TRT did not display AN in the subsequent voluntary intake test. That is, both the groups exposed to vinegar and those exposed to water during the previous TRT sessions decreased vinegar intake during the first voluntary drinking session. This suggests that voluntary intake might be essential in order to identify and process flavors – at the very least, intraoral infusion (i.e., involuntary exposure) had no effect on AN here. It might be argued that the absence of AN in the Experimental group was due to an aversive association between the flavor and the intraoral infusion procedure during TRT, but this would have been reflected in a delayed AN during voluntary intake test in comparison with Control group. Alternatively, it may be the case that the change of context imposed by the shift from passive infusion of the vinegar solution during the TRT phase, to active consumption in the test phase, may have interfered with any acquired familiarity. In this light, there is evidence that latent inhibition effects are attenuated by changes between passive exposure and active consumption (Fouquet et al., 2001; López et al., 2010; Yamamoto et al., 2002).

In addition, in Experiment 2 females displayed a lower number of appetitive responses than males which is also opposite to previous reports using sucrose, NaCl and quinine (Clarke & Ossenkopp, 1998; Flynn et al., 1993). Female rats also increased TRT aversive reactions to both vinegar solution and water in the second day, when the Experimental

group was exposed for first time to the vinegar solution, but this also happened in the Control group which still exposed to water. These were reduced progressively, presumably as they habituated to the infusion procedure. They also exhibited lower appetitive responses than males. It is conceivable that TRT results reflect females being more sensitive than males to aversive situations such as the intraoral infusion (perhaps because the volume of solution infused is relatively higher in females given their lower overall size). This would be also consistent with the higher reactivity described in females (Clarke & Ossenkopp, 1998; Flynn et al., 1993). It is important to note that appetitive and aversive reactions are mutually exclusive, so the high number of aversive reactions displayed by females is consistent with the small number of appetitive reactions. Although Fig. 2B and C may suggest higher aversive reactions in female experimental than control groups, and little such difference in male animals, the inferential analysis did not support the presence of a sex by group interaction, nor an overall effect of group. In any case, even if there was a true tendency for higher aversive reactions in the experimental group, this would not reflect neophobic responses to vinegar given that the same tendency also appears on the water pre and post sessions.

The discrepancy in the sensitivity of the methods to assess palatability using different behavioral procedures is interesting. We found LCS to be more sensitive than TRT to assess the potential hedonic changes involved in AN. However, TRT has been reported to be more sensitive to assess the hedonic changes involved in flavor preferences (Riordan & Dwyer, 2019). As noted above, this may be related to the effects of active as opposed to passive flavor exposure. Indeed, the high intake induced by flavor preferences might lead to ceiling effects using LCS. Alternatively, the processes involved in learning that a previously unfamiliar flavor is safe may differ from learning that a flavor is associated with positive consequences. Thus, a novel flavor might have mild aversive properties that attenuate as it becomes familiar, but this may not also involve learning about the positive consequences of ingestion. An additional issue concerns the apparent necessity of voluntary consumption to induce AN in Experiment 2. Despite six previous forced exposures through infusion via an intraoral cannula during TRT assessment, both male and female rats exhibited a similar vinegar neophobia in the first consumption test in comparison with non-exposed groups. As noted previously, this may reflect the impact of a context change between passive infusion and active voluntary consumption. Indeed, it has been previously demonstrated that a context shift can modulate neophobia and its attenuation (De la Casa & Díaz, 2013; Grau-Perales, Levy, et al., 2019; Greiner & Petrovich, 2020). One relevant factor may be differences produced by the change from oral infusion to voluntary consumption affecting the perception of the vinegar odor via orthonasal and retronasal routes. However, it should be remembered that Neath et al. (2010) did find increased voluntary consumption of saccharin in groups previously exposed during TRT in comparison with those not so exposed (although they too did not see changes in TRT reactions related to neophobia or its attenuation). The discrepancy could be due to differences in the tastes and procedures used: in addition to a difference in solution (saccharin vs. vinegar – which, in addition to being sweet vs sour, also differ in the strength of their odor components), they used shorter TRT infusions (10 s) and had no water baseline stabilization period while we applied longer TRT sessions (5 min) and 3 days of habituation to the water deprivation schedule before the voluntary consumption assessment. Nonetheless, our results seem to suggest that voluntary drinking plays at least some role in evaluating the consequences of ingesting novel tastes.

Returning to the general issue of sex differences, females showed higher reactivity than males in both palatability tests. They exhibit greater LCS than males in response to the familiar vinegar solution in Experiment 1. In Experiment 2 they exhibited changes of the aversive reactions along the TRT sessions while males did not. High female reactivity to sweet, salty and sour tastes has been previously found assessing the licking rate (Curtis et al., 2004) and TRT (Clarke & Ossenkopp, 1998; Flynn et al., 1993). Our results extend these findings to acid tastes. A discrepancy between previous TRT reports and our results, however, merits discussion. While increased female appetitive orofacial responses have been reported to sucrose, quinine (Clarke & Ossenkopp, 1998) and low NaCl concentration (Flynn et al., 1993) solutions, in the Experiment 2 females displayed fewer appetitive reactions than males – albeit not in a manner related flavor neophobia. As noted previously, this may be a consequence of the high levels of aversive TRT responses in female rats (alongside the fact that appetitive and aversive reactions are mutually incompatible). Further research is needed at this point in order to determine the processes involved in AN and the impact of sex differences.

Moreover, sex differences in consumption were observed in Experiment 1 such that AN after six vinegar exposures was complete in females but not in males (AN was not complete in either males or females in Experiment 2, although the difference between baseline water consumption and terminal levels of vinegar consumption was smaller in females than males). However, in a previous study (Expósito et al., 2023) the use of the same vinegar solution and a similar procedure did not find sex differences in AN. That said, there is at least one other report of sex differences in AN: using solid food and sweet taste (high-sucrose pellets vs. usual chow) male rats showed faster and more complete AN than females, especially when tested in a novel context (Greiner & Petrovich, 2020). In summary, the few examinations of sex effects in AN show a variety of results, including more complete/faster AN in females than males, faster AN in males than females, or no sex differences. Given the differences in the identity and nature (solid vs liquid) of the foods used, and differences in measurement techniques (consumption alone vs affective measures) it is difficult to determine an overall pattern to these results save to say that the presence or absence of sex differences in AN remains to be established and may depend on the exact circumstances of the test.

Finally, the absence of differences between *cacna1c* HET and WT rats in LCS-measured hedonic responses to unpalatable vinegar contrasts with the previous observation of reduced hedonic reactions to palatable sucrose in HET animals (Gasalla et al., 2025). That is, the effect of *cacna1c* on hedonic reactions appears to be a selectively anhedonic response to positive stimulation rather than a general emotional blunting. While we would assume that the results of *cacna1c* manipulation reflect differential expression in the brain between HET and WT animals, it should be remembered that *cacna1c* is expressed outside the brain and so peripheral contributions are also possible. Regardless, in the absence of a difference between HET and WT animals in the response to AN produced by vinegar exposure, the issue is moot here.

In summary, the present study examined the processes involved in taste neophobia and its attenuation using both intake and hedonic measures. The comparison of the affective LCS and TRT methods, which differ in their reliance on voluntary consumption vs passive exposure, suggests that voluntary consumption may play an important role in neophobia reduction given the AN effect was present only in Experiment 1 using LCS and voluntary consumption, but not with the TRT and oral infusion in Experiment 2. Comparison between male and female animals suggests that the general phenomena of AN is present in both consumption and hedonic reactions for both sexes, but that the exact pattern of consumption and hedonic change during AN may differ between them. While the fact that *cacna1c* HET and WT animals showed similar patterns of responding in AN (both consumption and hedonic measures) suggests that this genetic risk is selectively related to anhedonia revealed by reductions in positive reactions to normatively palatable solutions as opposed to reflecting a general insensitivity to palatability.

CRediT authorship contribution statement

S. Menchén-Márquez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Valero:** Investigation. **P. Gasalla:**

Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **F. Gámiz:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **M. Gallo:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **D.M. Dwyer:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethical statement

This study received was conducted following the local ethic guidelines, the UK Animals (Scientific Procedures) Act 1986 (project license number PP3468526) for the first experiment, and the ethical approval by the University of Granada Ethics Committee for Animal Research and by the Regional Ministry of Agriculture, Fisheries and Rural Development of Andalusia (1/06/2022/078) for the second experiment.

Declaration of competing interest

There are no competing interests to declare.

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Abbreviations

AN: Attenuation of neophobia; CTA: Conditioned taste aversion; CFP: Conditioned flavor preferences; TRT: Taste reactivity test; LCS: Lick cluster size (used as abbreviation of "analysis of licking microstructure" too); HET: Heterozygous; WT: Wild type; Vin: Cider vinegar; MLM: Mixed linear model; Exp: Experimental group.

Data availability

Data will be made available on request.

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