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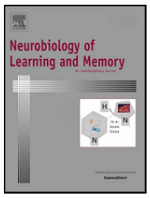

Perirhinal cortex supports both taste neophobia and its attenuation

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ABSTRACT

Rats are often reluctant to consume novel tastes because they lack knowledge about the postingestive effects the new foods might have. This paper examines the effect of excitotoxic lesions and temporary inactivation of the perirhinal cortex (Prh), a key region in the recognition memory system, on taste neophobia and its attenuation. Using a two-bottle choice paradigm (saccharin vs water), excitotoxic lesions were found to disrupt taste neophobia to 0.3% and 0.5% saccharin. However, the lesions had no effect when using a concentration of 0.7%, which is qualitatively aversive (expt. 1a-1c). In a second series of experiments the same animals were able to acquire a flavor preference learning on the basis of a flavor-taste association. Lesioned and control rats showed, during a choice test, a clear preference for the flavor associated with saccharin (expt. 2a-2c). Finally, in a third series of experiments, Prh inactivation with lidocaine after trial 1 (expt. 3) and after trials 1-3 (expt. 4) delayed attenuation of the neophobia. These findings suggest that Prh lesions do not significantly affect taste processing/ perception. Prh thus appears to play an essential role in taste neophobia and its attenuation.

1. Introduction

Animals have developed several mechanisms to protect themselves from potentially toxic/poisonous foods they find in their environment. One of these mechanisms is taste neophobia, which limits the ingestion of novel foods and thereby increases the chances of survival in case the novel food consumed is toxic (Corey, 1978; Domjan, 1976; Miller & Holzman, 1981; Reilly, 2018a). If the intake of the novel food is not followed by aversive consequences, then neophobia diminishes and intake will increase in subsequent encounters with the food (attenuation of the neophobia) and the taste will become familiar and be considered safe (Bermúdez-Rattoni, 2004; Best, Domjan, & Hanskins, 1978; Monk, Rubin, Keene, & Katz, 2014; Siegel, 1974). However, if after consuming the novel food subjects experience aversive post-ingestive consequences, they will develop conditioned taste aversion (CTA) and show reduced intake of the food in future encounters with it (Bernstein, 1999; Bures, Bermúdez-Rattoni, & Yamamoto, 1998; García, Kimmeldorf, & Koelling, 1955; Milgram, Krames, & Alloway, 1977; Reilly & Schachtman, 2009).

Recent research on the neurobiological substrates of taste neophobia has identified some structures that appear to be involved in the normal occurrence of this phenomenon (Osorio-Gómez, Guzmán-Ramos, & Bermúdez-Rattoni, 2018; Reilly, 2018b). Excitotoxic lesions to the gustatory thalamus or lesions to the medial amygdala disrupted taste neophobia and experimental rats consumed significantly more novel saccharin in trial 1 than control subjects, with both groups reaching a similar and asymptotic intake in trial 3-4 (Arthurs, Lin, & Reilly, 2018; Arthurs & Reilly, 2013; Lin, Roman, Arthurs, & Reilly, 2012; Lin, Roman, St. Andre, & Reilly, 2009). A greater impairment in taste neophobia has been observed following excitotoxic lesions to the basolateral amygdala or after lesions to the gustatory cortex, two structures that may be a functional unit (Lin & Reilly, 2012). Rats with bilateral excitotoxic lesions to either of these structures show a deficit pattern similar to the one observed following damage to the gustatory thalamus, that is, high intake of the novel saccharin solution (trial 1) and an intake similar to that of controls at asymptote by trial 3–4 (Dunn & Everitt, 1988; Lin et al., 2009; Moraga-Amaro, Cortés-Rojas, Simon, & Stehberg, 2014; Reilly, 2018b; Stehberg, Moraga-Amaro, & Simon, 2011). The temporary inactivation of either of the two structures mentioned (by infusion of baclofen/muscimol) before exposure to a novel saccharin solution induced both a deficit in taste neophobia in trial 1 and, surprisingly, a lower intake in trial 2 (Lin, Arthurs, & Reilly, 2018; see also, Nachman & Ashe, 1974; Gutiérrez, Téllez, & Bermúdez-Rattoni, 2003; Figueroa-Guzmán & Reilly, 2008). The absence of neophobia attenuation in trial 2 suggests that the pharmacological inactivation of these structures during the first encounter with a novel taste, and during the subsequent post-intake interval, delays the consolidation of the novel taste as a safe taste memory. This is important because it suggests that a single structure, in this case either the basolateral amygdala or the gusta-

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tory cortex, may be involved in two processes, one related to the initial neophobic response and one related to the plastic changes underlying neophobia attenuation (see also, Ho et al., 2015).

Since the ability to process/recognize a stimulus as either novel or familiar is essential for taste neophobia to occur normally, the present study aimed to investigate the contribution of the perirhinal cortex (Prh), a key region in recognition memory, to taste neophobia and its attenuation (Brown, Warburton, & Aggleton, 2010; Eichenbaum, Yonelinas, & Ranganath, 2007). There are several reasons to think that Prh may be involved in taste neophobia. First, neuroanatomical research has shown strong reciprocal connections between the Prh and the basolateral amygdala and the insular cortex, two regions associated with the initial neophobic response and its attenuation (Agster & Burwell, 2009; Agster, Pereira, Saddoris, & Burwell, 2016; Burwell & Amaral, 1998; Pereira, Agster, & Burwell, 2016; Shi & Cassel, 1999; Suzuki & Amaral, 1994). Therefore, Prh could interact critically with these structures during taste neophobia. Second, behavioral studies have established that Prh plays an essential role when animals show preferential exploration of novel vs familiar stimuli during a typical object recognition task (Albasser, Poirier, & Aggleton, 2010; Brown & Banks, 2015; Bussey, Muir, & Aggleton, 1999; Ennaceur, Neave, & Aggleton, 1996; Mumby & Pinel, 1994; Otto & Eichenbaum, 1992; Winters, Forwood, Cowell, Saksida, & Bussey, 2004; Winters, Saksida, & Bussey, 2008). Third, thanks to diverse techniques for recording/imaging neuronal activity, it has been possible to observe that many neurons in Prh and its neighboring regions respond more intensely to novel than to familiar stimuli. This effect is known as "repetition suppression" and has been observed in multiple studies (Ahn, Lee, & Lee, 2019; Brown, 2000; Fahy, Riches, & Brown, 1993; Li, Miller, & Desimone, 1993; von Linstow Roloff, Muller, & Brown, 2016; Wan, Aggleton, & Brown, 1999; Xiang & Brown, 1998; Young, Otto, Fox, & Eichenbaum, 1997; Zhu, Brown, & Aggleton, 1995). Although most of the studies have used visual stimuli, a recent report has confirmed the same effect with gustatory stimuli. More specifically, using c-Fos immunoreactivity as a marker for neuronal activity, its authors showed that intake of a novel flavor solution induced a higher number of Prh c-Fos-positive neurons during trial 1 than during trial 2 and trial 6 (Gómez-Chacón, Morillas, & Gallo, 2015). Finally, a change in activity opposite to the aforementioned one –that is, an increase in activity due to the repetition of the stimulus– has been found less frequently within the Prh and has been associated with the plastic changes underlying memory consolidation (see, for example, Hölscher, Rolls, & Xiang, 2003).

In accordance with the foregoing, a previous study in our laboratory found that excitotoxic lesions to the Prh impaired taste neophobia. Thus, Prh-lesioned rats drank significantly more novel 0.3% and 0.5% saccharin solution than control subjects in trial 1, although the amount of fluid intake at asymptote was similar in the two groups in trial 2-3 (Ramos, 2015). This study, however, raises a series of important issues, further study of which is needed to help clarify Prh contribution to taste neophobia. The first aim of this study was to replicate our previous results, but on this occasion a two-bottle choice test was used each day (from trial 1 to trial 4) and the rats had to discriminate between water vs a saccharin solution (experiments 1a, 1b and 1c). We hypothesized that Prh-lesioned rats would drink more saccharin than the controls in trial 1, thereby replicating our previous data in a two-bottle choice test procedure. Second, to examine whether Prh-lesioned rats have a gustatory perceptual deficit, the same rats used in the three previous experiments were subjected to a flavor-taste preference learning task. The aim was to investigate whether lesioned rats could discriminate as well as controls between two flavored solutions with different hedonic value (experiments 2a, 2b and 2c). Third, to determine whether Prh also contributes to a novel taste being deemed safe and familiar, we examined the effect of lidocaine infusion into the Prh immediately following the intake of a saccharin solution (experiments 3 and 4). We found that Prh is critical for both the initial taste neophobia response and its attenuation.

2. Materials and methods

2.1. Experiments 1a, 1b and 1c: Prh-lesions disrupt taste neophobia when using a two-bottle choice procedure (saccharin versus water)

Our main aim was to replicate the previous findings showing impairment in taste neophobia after Prh lesions (Ramos, 2015). However, in the present study we used a two-bottle choice procedure (saccharin versus water) instead of a one-bottle test. Using a two-bottle test, a disruption in the neophobia would easily be detected if lesioned rats in trial 1 drank approximately the same amount of saccharin as water and showed a preference for saccharin significantly greater than that observed in the control group. The two groups should, however, show a similar intake and preference for saccharin at asymptote.

2.1.1. Subjects

The subjects were 44 male Wistar rats from Charles River Laboratories (France). Specifically, the number of animals per experiment was (n lesioned vs n controls): experiment 1a = 8 versus 7; experiment 1b = 8 versus 6; experiment 1c = 8 versus 7. The rats initially weighing between 270 and 290 g were individually housed in single polycarbonate cages (480 × 265 × 210 mm, Tecniplast, Italy) and maintained at a constant temperature of 22 ± 1 °C. Rats were given *ad libitum* food and water until experiments started. All experimental procedures were performed in conformity with European (2010/63 EEC) and Spanish (BOE RD 53/2013) legislation and were approved by the Ethics Committee for Animal Research of the University of Granada.

2.1.2. Surgery

Under the effects of sodium pentobarbital anesthesia (60 mg/kg, i.p., Sigma Chemical, St. Louis, Missouri), the rats were placed in a David Kopf stereotaxic apparatus (mod. 900, David Kopf Instruments, Tujunga, California) with the incisor bar adjusted so that lambda and bregma were level. Rats were randomly assigned to either an experimental or a control group. The lesioned subjects received bilateral injections of N-methyl-D-aspartic acid (NMDA, Sigma Chemical, PBS, pH 7.4, 0.07 M) through the insertion of a 30-gauge stainless steel cannula in six sites of the perirhinal cortex. The cannula was laterally oriented at 26° from the vertical. The coordinates were derived from the atlas of Paxinos and Watson (1998) and based on the anatomical location of the perirhinal cortex, as delineated by Burwell and colleagues (Burwell, 2001; Burwell & Amaral, 1998; Burwell, Witter, & Amaral, 1995; Furtak, We, Agster, & Burwell, 2007). The anteroposterior (AP) stereotaxic coordinates were calculated relative to bregma, the lateral (L) relative to the midline and the dorsoventral (V) relative to the top of the skull: AP = −3.6, L = ±2.9, V = 9.8; AP = −4.8, L = ±3.3, V = 9.8; AP = −5.8, L = ±2.8, V = 9.8. NMDA was administered in a 0.3 μl volume at each site through the cannula that was attached to a 5 μl Hamilton microsyringe (Teknokroma, Barcelona, Spain). Delivery of the solution was carried out with a Harvard Apparatus pump set (model 22, Panlab-Harvard Apparatus, Barcelona, Spain) at an infusion rate of 0.1 μl/min. The cannula was left *in situ* for an additional 5 min before being withdrawn. The control groups underwent identical surgical procedures with one exception, that equivalent volumes of phosphate-buffered saline (PBS) were infused into the Prh. After surgery, each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, richterpharma, ag, Austria).

2.1.3. Behavioral procedure

All behavioral testing occurred in the home cages. After recovery from surgery, rats were adapted to a water-restriction schedule of 15-min of water access in the morning followed 6 h later by a second 15-min period of water access in the afternoon. After 4 days of this habituation program, on the fifth day, taste neophobia trials began. Dur-

ing each of the neophobia trials (from day 5 to day 8) the animals were presented, in the middle of the front part of their home cage, with two calibrated tubes fitted with a rubber stopper and a steel sipper spout extending 1.5 cm into the cage. The two tubes were 2 cm apart from each other. One tube contained water and the other tube contained a sodium saccharin solution (Sigma-Aldrich, Madrid, Spain) at 0.3% (experiment 1a), 0.5% (experiment 1b) or 0.7% (experiment 1c). The position of the tubes was doubly counterbalanced, by day and over the course of the days, to control for possible side preferences. The tastant was always presented in the morning. Saccharin and water intake was assessed by weighing the tubes before and after each neophobia trial, to the nearest 0.1 g. Six hours after each neophobia trial, all rats had unlimited access to water for 15 min.

2.1.4. Data analysis

The preference for saccharin over water was analysed using a 2-way mixed design analysis of variance (ANOVA) with group as the between-subject variable and trial as the within-subject variable (2 group × 4 trial). The saccharin preference index, defined as the percentage of saccharin consumed in relation to the total fluid consumed in each taste neophobia trial, was calculated with the following formula (see Bahar, Dorfman, & Dudai, 2004):

$$\text{Preference index} = 100 \times \text{saccharin consumed}/(\text{water consumed} + \text{saccharin consumed})$$

Hence, a preference index >50 implies higher preference to saccharin over water. Post-hoc Tukey tests were used where appropriate. All analyses were conducted with the Statistica software 8.0 (StatSoft, Tulsa, Oklahoma).

2.2. Experiments 2a, 2b and 2c: Effect of Prh lesions on conditioned flavor preference based on a flavor-taste learning paradigm

These experiments were designed to rule out the possibility that the deficit in taste neophobia seen in rats with Prh lesions could be due to an alteration in taste processing. Specifically, the aim was to examine whether the lesioned rats of the three previous experiments were capable of discriminating between two flavored solutions using a flavor-taste learning paradigm. The acquisition of flavor preferences seems to depend on associative learning mechanisms (Díaz & De la Casa, 2011; Díaz, De la Casa, Ruiz, & Baeyens, 2004; Rozin & Zellner, 1985). Two types of flavor preference can be established in laboratories: flavor-nutrient and flavor-taste conditioning. In the former, two neutral flavors are presented every other day for several days, pairing one of them (CS+) with post-ingestive consequences. In the latter, of the two initially non-preferred flavors, one is paired (CS+) every other day with a sweet taste that has no postingestive effect. Many studies have shown that after several days of pairing, when a two-bottle choice test is used in which both flavors are simultaneously presented in water, normal rats prefer the taste that was paired with postingestive consequences or the sweet taste, respectively (Dwyer, 2011; Reilly & Pritchard, 1996; Sclafani, 2004; Sclafani & Ackroff, 1994; Touzani & Sclafani, 2005; Ueji, Minematsu, Takeshita, & Yamamoto, 2016; Yamamoto & Ueji, 2011).

With regard to flavor-taste conditioning, some studies have shown robust learning using saccharin as the sweet taste without post-ingestive/nutritional consequences (Díaz & De la Casa, 2011; Díaz et al., 2004; Fanselow & Birk, 1982; Forestell & Lolordo, 2003; Holman, 1975; Messier & White, 1984; Ueji et al., 2016). Therefore, since the flavor-taste conditioning using saccharin as US depends completely on orosensory mechanisms, this procedure seems ideal for evaluating whether Prh-lesioned rats have a deficit in taste processing/perception. We hypothesized that if such a deficit existed, lesioned rats would fail when discriminating between the two flavors (CS+ versus CS−) and impairment in flavor-taste conditioning would be evident. On the other hand, normal flavor-taste preference learning would be the sign of no deficit in taste processing/perception.

2.2.1. Subjects

The subjects were the same as the ones used in experiments 1a, 1b and 1c.

2.2.2. Behavioral procedure

After completing experiments 1a, 1b and 1c the rats were maintained *ad libitum* in their usual cages for 14 days. Then the animals were adapted, over a period of 4 days, to a water-restriction schedule like the one used in the first series of experiments. During the following six days (days 5–10) the acquisition phase took place, to establish a flavor-taste preference learning using a modified version of procedures described in previous studies (Dwyer, 2011; Gilbert, Campbell, & Kesner, 2003; Renteria, Silbaugh, Tolentino, & Gilbert, 2008; Ueji et al., 2016). During these six consecutive days, half of the rats in the lesioned and control groups were presented with an unsweetened grape-flavored solution (CS-w) on odd days and with a sweetened cherry-flavored solution (CS+ saccharin) on even days. The other half of the rats received a sweetened grape-flavored solution (CS+ saccharin) on odd days and an unsweetened cherry-flavored solution (CS-w) on even days. The CS+ solutions were sodium saccharin (0.175% w/w, Sigma-Aldrich, Madrid, Spain) flavored with grape or cherry Kool Aid (0.05%, Kraft Foods, NY, USA). The CS− were the flavored solution, grape or cherry, but they were not mixed with saccharin. In a choice between these flavors, naïve rats show no preference whatsoever (Elizalde & Sclafani, 1990). The CS+ or the CS− were presented for 15 min in the morning session. The tubes were placed in the middle of the front part of the cage. Six hours after each training trial, all rats had unlimited access to water for 15 min. Since we observed that rats drank a slightly greater quantity of CS+ than of CS− during the 6 days of training, all animals had a seventh additional day of training in order to make the magnitude of the CS+ and CS− consumed by each subject exactly equal. Thus, on day 11, during the 15 min session in the morning, each rat was presented with a tube that contained its unsweetened solution (CS−) in the volume required to equal the amount of CS+ consumed during the training. Once each animal had consumed the additional unsweetened solution, it was presented with a tube with water and had unlimited access during the remainder of the 15 min session. In this way, during the training all the animals consumed the same amount of both solutions, CS+ and CS−. On day 12 the choice-preference test was performed and each rat was presented simultaneously with two graduated tubes in the middle of the front part of the cage, 2 cm apart from each other. One tube contained an unsweetened grape-flavored solution and the other contained an unsweetened cherry-flavored solution (both at 0.05%), with the position of each flavor being counterbalanced in each group. The amount of each solution consumed was recorded to the nearest 0.1 g.

2.2.3. Histology

When the behavioral testing was completed, the rats were deeply anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and perfused intercardially with 0.9% saline, followed by 10% formalin. After extraction from the skull, the brains were post-fixed in 10% formalin for several days and subsequently in 10% formalin-30% sucrose until sectioning. Coronal sections (50 μm) were cut on a cryostat (Leica CM 1850, Leica Microsystems, Germany) and stained with Cresyl violet, a Nissl stain.

In order to quantify the extension of the damage in each lesioned rat, regions of cell loss and gliosis identified microscopically were plotted on drawings of coronal sections from the Paxinos & Watson atlas (1998). For each perirhinal-lesioned rat, the reconstruction of the lesion was made based on eight coronal sections (anteroposterior levels from bregma: −3.3, −3.8, −4.3, −4.8, −5.2, −5.6, −6.0 and −6.3 mm). Each coronal section was digitized and the lesioned area was calcu-

lated (ImageJ, http://imagej.nih.gov/ij/). The anatomical limits of the perirhinal, entorhinal and postrhinal cortices were defined using works by Burwell and associates (Burwell, 2001; Burwell et al., 1995). The volume of damage was expressed as a percentage, reflecting the amount of lesioned tissue in relation to four normal non-lesioned rats.

2.2.4. Data analysis

During the acquisition phase, the intake of the sweetened solution and the unsweetened solution over the 6 days of training was analysed with a 3-way mixed design analysis of variance (ANOVA), with group as the between-subject variable, trial as a within-subject variable and solution as another within-subject variable (2 group × 3 trial × 2 solution). During the two-bottle choice-preference test, which took place on experimental day 12, the preference of one flavor over another was analysed using a 2-way mixed design analysis of variance (ANOVA) with group as the between-subject variable and solution as the within-subject variable (2 group × 2 solution). Post-hoc Tukey tests for the analyses of simple main effects were used where appropriate.

2.3. Experiment 3: Prh lidocaine inactivation immediately after trial 1

Since in the previous experiments permanent excitotoxic lesions only impaired the initial neophobic response, the aim of experiments 3 and 4 was to investigate whether Prh is involved in the attenuation of taste neophobia. To selectively affect the memory formation process, in these experiments Prh was temporarily inactivated immediately after taste presentation. In experiment 3 transient inactivation of Prh was accomplished immediately after the first taste neophobia trial by means of bilateral microinfusions of lidocaine, a sodium channel blocker with short-lived effects (Martin, 1991; Pereira de Vasconcelos et al., 2006; Tehovnik & Sommer, 1997). Previous studies have shown that in low amounts and low doses, lidocaine locally infused into Prh disrupts consolidation of object recognition memory when it is applied after the sample phase (Winters & Bussey, 2005). Consequently, if Prh is involved in taste neophobia attenuation, the reversible blocking of the perirhinal region immediately after the intake of the saccharin solution should disrupt this process, delaying the transition from novel to familiar safe taste.

A central objective of experiments 1a-1c was to repeat our previous data, obtained with one-bottle forced-drinking protocol (see, Ramos, 2015), but using a different protocol. For this reason a two-bottle procedure was used in experiments 1a-1c. Equivalent conclusions have been obtained using one-bottle forced-drinking and two-bottle choice procedures. So, in experiments 3 and 4 we used the one-bottle procedure which is commonly used as a standard taste neophobia protocol in the studies conducted in this area (Reilly, 2018a).

2.3.1. Subjects

The subjects were 16 male Wistar rats from Charles River Laboratories (France), with the same characteristics as those described in the earlier experiments.

2.3.2. Surgery

Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (15 mg/kg) and placed in a David Kopf stereotaxic apparatus with the incisor bar adjusted so that lambda and bregma were level. All animals were implanted bilaterally with 22-gauge stainless steel guide cannulas built in our lab. Each cannula was laterally oriented at 26° from the vertical and implanted using the following coordinates: AP = -4.4 mm from bregma; L = ±3.3 mm from the midline; V = 4 mm from the skull surface (Paxinos & Watson, 1998). The cannulas were anchored to the skull by two stainless steel screws and dental cement and closed with a dummy cannula to maintain patency. Each rat received the antibiotic Omnamicina (0.1 cc intramuscular; Hoechst Ibérica, Spain) and buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, richterpharma, ag, Austria). Rats were given at least 10 days of recovery before training began.

2.3.3. Behavioral procedure

After recovery from surgery rats were adapted over a period of 4 days to a water-restriction schedule like the one used in the previous experiments. During these four days the animals were habituated to the infusion process, as follows: after the 15 min period of water access in the morning, each rat was taken to the experimental room where the intracranial microinfusions were to take place and was immediately taken from its cage and restrained gently by the experimenter for 3 min. The dummy cannulas were removed during this time and the rats could hear the sound of the infusion pump. After this period of 4 days the taste neophobia trials began, which lasted 3 consecutive days (from day 5 to day 7). The behavioral procedure was the same as used in experiments 1a-1c, with the exception that now in each taste neophobia trial only one calibrated tube was presented in the middle of the front part of each animal's cage. The tube contained a sodium saccharin solution at 0.5%. A taste neophobia trial consisted of 15-min access to the saccharin solution during the morning period. On experimental day 5, right before the infusion of lidocaine or buffer into the Prh, each animal was randomly assigned to either the experimental or the control group without taking into account the number of animals that had already been assigned to one group or the other just moments before (experimental n = 6, control n = 10). All rats received bilateral intracranial microinfusions 1–2 min after trial 1. Experimental rats received lidocaine hydrochoride at 4% (Sigma-Aldrich, Madrid; 40 μg/μl in PBS; 1 μl in 90 s per side) and control rats received buffer infusions. Prior to the infusion, dummy cannulas were removed and infusion cannulas (30-gauge stainless steel), measuring 9.7 mm in length from the surface of the skull to the target region, were inserted into the guide cannula. Bilateral microinfusions were made simultaneously, using two 5-μl Hamilton microsyringes (Teknokroma, Barcelona). The microsyringes were driven by a Harvard Apparatus pump (model 22, Panlab, Barcelona) and the cannulas were left *in situ* for an additional 2 min before being withdrawn.

2.3.4. Histology

At the end of the experiment all rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p. Sigma Chemical, St. Louis, Missouri) and placed in a David Kopf stereotaxic apparatus with the incisor bar adjusted so that lambda and bregma were level. The same infusion cannulas used previously for the intracranial microinjections were inserted now in the guide cannulas of each rat, to produce small excitotoxic lesions in the target zone, in order to more precisely evaluate placement of the injection tip. All rats received bilateral injections of NMDA (Sigma Chemical, PBS, pH 7.4, 0.12 M), administering 0.05 μl of the neurotoxin in each hemisphere. The injection cannulas were attached a 5-μl Hamilton microsyringe and the delivery of the solution was carried out with a Harvard Apparatus pump set at an infusion rate of 0.1 μl/min. The cannula was left *in situ* for 3 min before being withdrawn. Ten days later all animals were sacrificed by an overdose of sodium pentobarbital and perfused intercardially with 0.9% saline, followed by 10% formalin. The brains were post-fixed in 10% formalin for several days and subsequently in 10% formalin-30% sucrose, and then sectioned and stained with Cresyl violet to study the injection tip placement under a microscope.

2.3.5. Data analysis

The intake of saccharin was analysed with a 2-way mixed design analysis of variance (ANOVA) with group as the between-subject variable and trial as the within-subject variable (2 group × 3 trial). A 2-way mixed ANOVA was also used to compare the volume of water consumed on the last day of the water-restriction schedule with the saccharin consumed during the first taste neophobia trial (day 4 vs day 5,

2 group × 2 trial). Post-hoc Tukey tests were used for the analyses of simple main effects.

2.4. Experiment 4: Prh lidocaine inactivation immediately after trial 1, 2 and 3

To determine whether in the previous experiment Prh lidocaine infusion actually disrupts taste neophobia attenuation or simply induces taste aversion learning by acting as an unconditioned stimulus, in the present experiment we repeated experiment 3 but on this occasion lidocaine infusion was applied immediately after trial 1, 2 and 3. It has been established in studies elsewhere that an increase in the number of trials pairing CS-US increases the intensity of the CTA (Bures et al., 1998; Riley & Mastropaolo, 1989; Roman, Lin, & Reilly, 2009; Roman & Reilly, 2007). Therefore, if Prh lidocaine infusion induces CTA, three CS-US trials, taking place on three consecutive days, should produce a significantly greater reduction in the consumption of saccharin than the one observed in experiment 3, in which only one CS-US trial was applied.

2.4.1. Subjects

The subjects were thirteen male Wistar rats like the ones described in previous experiments. Six rats were assigned randomly to the experimental group and seven to the control group.

2.4.2. Surgery

As described in experiment 3.

2.4.3. Behavioral procedure

The behavioral procedure was identical to the one described in experiment 3 except on this occasion three intracortical infusions were applied in the Prh immediately after trial 1, 2 and 3 (days 5, 6 and 7) and six neophobia trials were conducted (from day 5 to day 10).

2.4.4. Histology

The procedures were the same as those followed in experiment 3.

2.4.5. Data analyses

Two 2-way mixed analysis of variance (ANOVA 2 × 2 and 2 × 6) with group as between-subject variable and trial as within-subject variable were used where appropriate. Post-hoc Tukey tests were used for the analyses of simple effects.

3. Results

3.1. Experiments 1a, 1b and 1c

3.1.1. Histological results

Representative photomicrographs and a diagram representing the extension of the lesions of the experimental subjects, which were the same in experiments 1a-1c and 2a-2c, are shown in Fig. 1. In general, the lesions affected all layers of the Prh and rarely reached the rostral portion of the postrhinal cortex or the CA1 field of the ventral hippocampus. Some rats presented minimal damage in the lateral entorhinal cortex and the ventral temporal association area, but such lesions were always under 10% of these structures. The insular cortex was intact in all the subjects (see Cechetto & Saper, 1987; Kosar, Grill, & Norgren, 1986). A one-way analysis of variance (ANOVA) indicated that the mean percentage of the perirhinal damage (areas 35 and 36) among the Prh-lesioned groups was similar ($F < 1$), ranging from 69 to 89 percent.

3.1.2. Behavioral results

3.1.2.1. Experiment 1a: 0.3% saccharin versus water To study the disruption of neophobia following Prh lesions we analyzed the data using the saccharin preference index as a dependent variable (Fig. 2A).

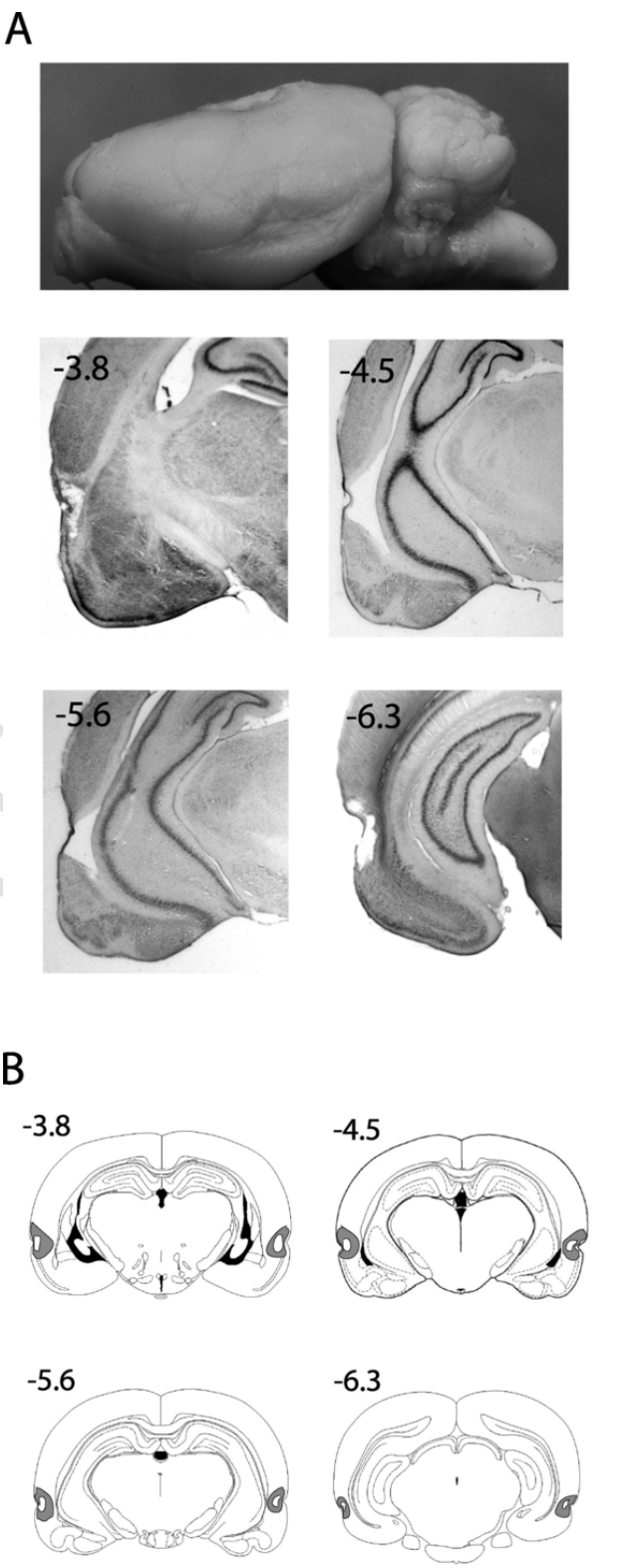

Fig. 1. A) Lateral view of a representative perirhinal lesion and photomicrographs of coronal sections stained with cresyl violet from a representative lesioned rat. B) Coronal sections showing the largest (gray) and smallest (central white area) perirhinal lesions in the lesioned groups of experiments 1a-1c and experiments 2a-2c. AP coordinates are shown in relation to the bregma.

Data indicated a clear neophobia in the control animals but a disruption in the neophobia in the lesioned group. Thus, in the first neophobia trial, Prh-lesioned rats drank very similar amounts of saccharin and wa-

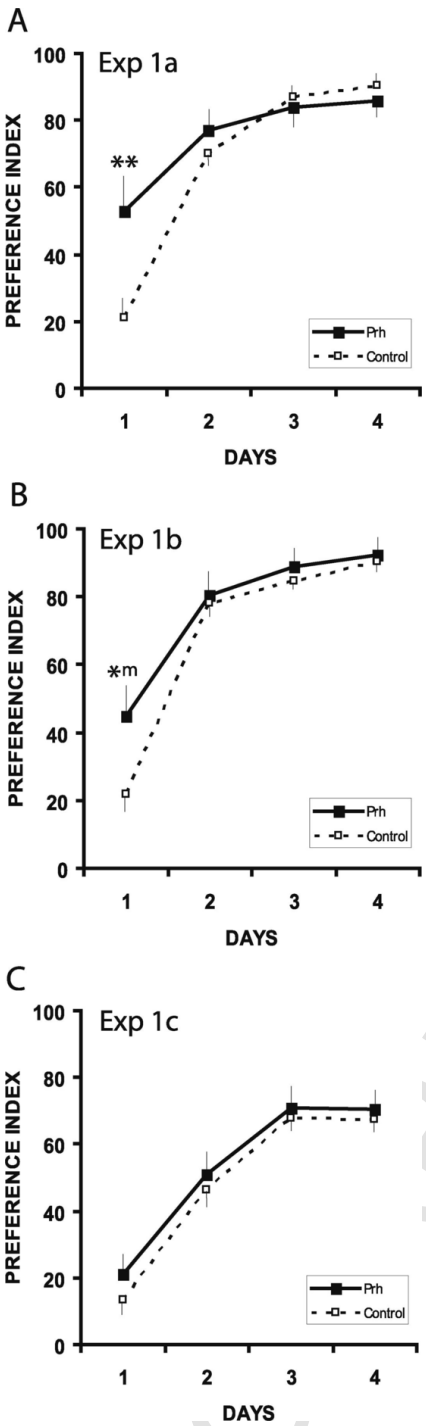

Fig. 2. Index of preference to saccharin (mean ± SEM) in experiment 1a (A), experiment 1b (B) and experiment 1c (C). ** $p < 0.01$, *m marginally significant.

ter (mean saccharin = 4.8 ± 1.1 versus mean water = 3.5 ± 0.6). This pattern contrasts with the performance of the control group, which consumed almost four times more water than saccharin (mean saccharin = 1.5 ± 0.4 versus mean water = 5.8 ± 0.7). A 2-way mixed design analysis of variance (ANOVA) showed no significant effect of group ($F_{1, 13} = 2.06$, $p = 0.17$, $\eta^2_p = 0.13$), a significant effect of trial ($F_{3, 39} = 40.60$, $p < 0.0001$, $\eta^2_p = 0.75$) and a significant group × trial interaction ($F_{3, 39} = 5.98$, $p < 0.001$, $\eta^2_p = 0.31$). Tukey tests to analyze the interaction revealed that Prh-lesioned animals presented a significantly higher preference for saccharin than controls did in the first trial ($p < 0.01$) but not in the second, third and fourth trial. This data suggests a disrupted neophobic response in Prh-lesioned rats. In addition, Tukey tests to analyze the trial factor showed that control rats presented a significantly lower preference index in the first trial compared to the second trial ($p < 0.0001$) but no significant differences were detected when comparing the intake of the second and third days ($p = 0.50$) and the intake of the third and fourth days ($p = 0.99$). Regarding the experimental group, significant differences were observed between trial 1 and 2 ($p < 0.03$) but no differences were found between trial 2 and 3 ($p = 0.98$) or between 3 and 4 ($p = 1.0$). This means that the neophobic response of both groups was attenuated in the second trial. Also, both groups presented asymptotic performance on the fourth and last day of the experiment.

3.1.2.2. Experiment 1b: 0.5% saccharin versus water Fig. 2B represents the saccharin preference index obtained in lesioned and control rats during the four consecutive trials of experiment 1b. A 2-way mixed design analysis of variance (ANOVA) showed no significant effect between groups ($F_{1, 12} = 1.81$, $p = 0.20$, $\eta^2_p = 0.13$), but a significant effect of trial ($F_{3, 36} = 104.09$, $p < 0.0001$, $\eta^2_p = 0.89$) and a significant group × trial interaction ($F_{3, 36} = 3.76$, $p < 0.01$, $\eta^2_p = 0.23$) were observed. The interaction analysis indicated a greater but marginal preference for saccharin in lesioned rats than in controls in trial 1 ($p = 0.07$) but a similar preference in trials 2–4 ($p = 0.99$). Furthermore, Tukey tests for the trial factor showed that control animals presented a significantly lower preference index in the first trial than in the second ($p < 0.0001$) but no significant differences were detected upon comparing the preference index of trial 2 with that of trial 3 ($p = 0.89$), or upon comparing the third and fourth trial ($p = 0.94$). With regard to the experimental group, significant differences were observed between trial 1 and 2 ($p < 0.0001$), but no differences were found between trial 2 and 3 ($p = 0.74$) or between trial 3 and 4 ($p = 0.98$). As in experiment 1a, these results support a deficit in the initial taste neophobic response in Prh-lesioned rats, but not a disruption in neophobia attenuation.

3.1.2.3. Experiment 1c: 0.7% saccharin versus water Fig. 2C shows the saccharin preference index obtained in lesioned and control rats during the four consecutive trials of experiment 1c. A 2-way mixed design analysis of variance (ANOVA) indicated that only the trial main effect was significant ($F_{1, 13}$ group = 0.45, $p = 0.51$, $\eta^2_p = 0.03$; $F_{3, 39}$ trial = 30.06, $p < 0.0001$, $\eta^2_p = 0.70$; $F_{3, 39}$ group × trial = 0.02, $p = 0.99$, $\eta^2_p = 0.08$). The analysis of the main trial factor revealed significant differences upon comparing trial 1 with trial 2 ($p < 0.001$) and trial 2 with trial 3 ($p < 0.01$), but asymptotic performance upon comparing trial 3 and trial 4 ($p = 0.99$). These data suggest normal taste neophobia and normal attenuation of the neophobia in lesioned and control rats.

3.2. Experiments 2a, 2b and 2c

3.2.1. Histological results

Representative Prh lesions are summarized in Fig. 1.

3.2.2. Behavioral results

3.2.2.1. Experiment 2a Fig. 3A shows the intake of CS+ and CS− during the acquisition. A 3-way mixed design ANOVA found significant results in the trial main effect ($F_{2, 26} = 5.99$, $p < 0.01$, $\eta^2_p = 0.31$) and in the solution main effect ($F_{1, 13} = 15.51$, $p < 0.001$, $\eta^2_p = 0.54$) but not in the group main effect ($F_{1, 13} = 0.45$, $p = 0.50$, $\eta^2_p = 0.03$) nor in the group × trial × solution main interaction ($F_{2, 26} = 1.90$, $p = 0.16$, $\eta^2_p = 0.12$). A secondary interaction was also significant ($F_{2, 26}$ trial × solution = 5.15, $p < 0.01$, $\eta^2_p = 0.28$).Fig. 3D depicts mean intakes of CS+ and CS− in the two-bottle choice-preference test. A 2-way mixed ANOVA (2 group × 2 solution) showed only a significant effect of the factor solution ($F_{1, 13} = 31.57$, $p < 0.0001$, $\eta^2_p = 0.70$), not of the factor group ($F_{1, 13} = 0.13$, $p = 0.71$, $\eta^2_p = 0.00$) nor of the interaction ($F_{1, 13} = 0.00$, $p = 0.95$, $\eta^2_p = 0.00$). Based on this, the size of the solution effect can be consid-

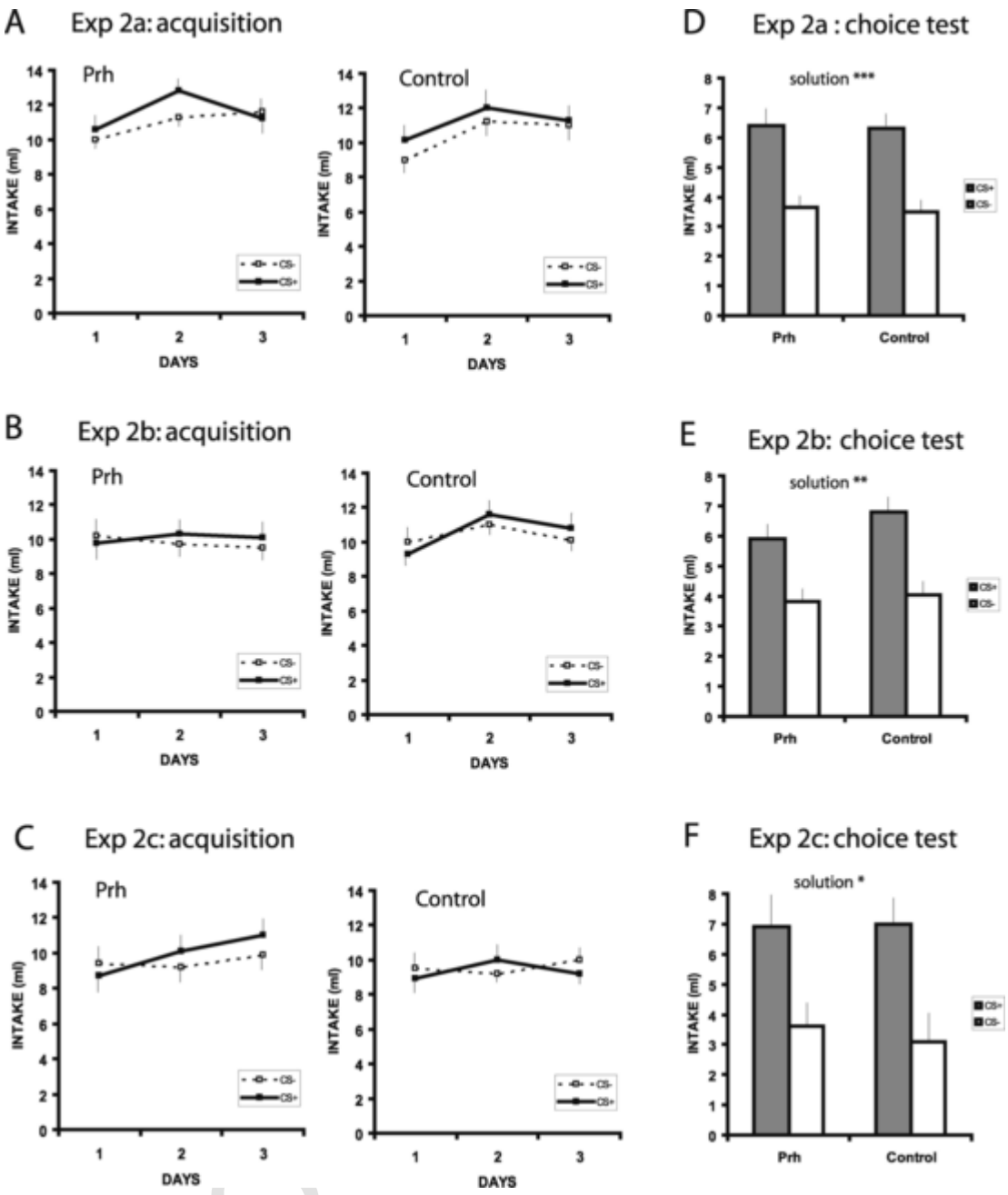

Fig. 3. A, B and C: Acquisition of flavor-taste preference learning in experiment 2a, 2b and 2c, respectively. Intake of CS+ and CS− flavors (mean ± SEM) across acquisition sessions. D, E and F: Mean intake (±SEM) of CS+ and CS− flavors in the choice-preference test in experiments 2a, 2b and 2c, respectively. *** $p < 0.0001$, ** $p < 0.001$, * $p < 0.01$.

ered equivalent in the lesioned and control groups. It appears that both groups acquired flavor-taste preference learning, discriminating finely between the two solutions (CS+ vs CS−) in the choice test.

3.2.2.2. Experiment 2b Results regarding the acquisition are represented in Fig. 3B. A 3-way mixed design ANOVA found significant results in the trial factor ($F_{2, 24} = 3.46$, $p < 0.04$, $\eta^2_p = 0.22$) and in the solution factor ($F_{1, 12} = 13.14$, $p < 0.01$, $\eta^2_p = 0.52$) but not in the group factor ($F_{1, 12} = 0.17$, $p = 0.68$, $\eta^2_p = 0.01$) nor in the group × trial × solution main interaction ($F_{2, 24} = 1.16$, $p = 0.84$, $\eta^2_p = 0.01$). As in experiment 2a, a secondary interaction was significant ($F_{2, 24}$ trial × solution = 18.31, $p < 0.0001$, $\eta^2_p = 0.60$).During the choice-preference test, a 2-way mixed ANOVA revealed only a significant effect of the factor solution ($F_{1, 12} = 26.49$, $p < 0.001$, $\eta^2_p = 0.68$) and not of the factor group ($F_{1, 12} = 3.23$, $p = 0.09$, $\eta^2_p = 0.21$) nor of the interaction ($F_{1, 12} = 0.58$, $p = 0.45$, $\eta^2_p = 0.04$. See Fig. 3E). As in the preceding experiment, these results imply that the size of the solution effect was equivalent in lesioned and control rats, suggesting normal flavor-taste preference learning in both groups.

3.2.2.3. Experiment 2c Fig. 3C depicts intake of the CS+ and CS− during the three acquisition trials. A 3-way mixed ANOVA found significant results only in the secondary interaction trial × solution ($F_{2, 26} = 3.34$, $p = 0.05$, $\eta^2_p = 0.20$).During the choice-preference test, a 2-way mixed ANOVA showed only a significant main effect of solution ($F_{1, 13} = 11.70$, $p < 0.01$, $\eta^2_p = 0.47$) and not of the factor group ($F_{1, 13} = 0.61$, $p = 0.44$, $\eta^2_p = 0.04$) nor of the interaction ($F_{1, 13} = 0.13$, $p = 0.71$, $\eta^2_p = 0.01$. See Fig. 3F). The finding that neither the factor group nor the interaction group × solution was significant implies that the size of the solution effect was equivalent in the two groups, sug-

gesting that both groups discriminated in the same way between the two solutions (CS+ vs CS−) available in the choice test.

3.3. Experiment 3

3.3.1. Histological results

Fig. 4 depicts the location of the tip of the injection cannulas for the experimental rats. In all the rats the infusion needle tips were within the perirhinal region, in accordance with the limits indicated by Burwell (2001).

3.3.2. Behavioral results

The behavioral results appear in Fig. 5A and 5B. The main results were the following. First, a 2-way mixed ANOVA showed that our procedure produced a robust gustatory neophobia. Thus, upon comparing the water intake on the last day of water access with that of saccharin in the first neophobic trial, only a significant effect of trial was found ($F_{1, 14}$ group = 0.01, p = 0.91, η^2_p = 0.00; $F_{1, 14}$ trial = 194.57, p < 0.0001, η^2_p = 0.93; $F_{1, 14}$ group × trial = 3.32, p = 0.09, η^2_p = 0.19). Second, upon analyzing the intake of saccharin over the course of the 3 neophobic trials, a 2-way mixed ANOVA showed a marginal group main effect ($F_{1, 14}$ = 3.40, p = 0.08, η^2_p = 0.19), a significant trial main effect ($F_{2, 28}$ = 39.97, p < 0.0001, η^2_p = 0.73) and a significant group × trial interaction ($F_{2, 28}$ = 7.04, p < 0.01, η^2_p = 0.33). Tukey tests for the analysis of the interaction revealed significant differences between experimental and control animals only in trial 2 (p < 0.01), and not in trial 1 (p = 0.99) nor in trial 3 (p = 0.87). In addition, post-hoc Tukey tests to analyze the trial factor showed normal attenuation of the neophobia in the control group but disrupted attenuation in the experimental group. Specifically, control subjects consumed significantly more saccharin during the second neophobic trial than during the first one (p < 0.0001), with saccharin intake stabilizing in the third trial (trial 2 vs trial 3, p = 0.52). In contrast, lidocaine-injected rats drank a similar amount of saccharin in trial 2 and trial 1 (p = 0.99) suggesting a deterioration in the neophobia attenuation. However, lidocaine-injected rats showed significantly greater intake in trial 3 than in trial 2 (p < 0.01). Importantly, Cohen's d effect size comparing trial 2 to trial 3 in experimental rats was 0.68. Taken together these data suggest that Prh neural activity is necessary, at least during a short time after the first experience with a taste, in order for normal attenuation of the neophobia to occur.

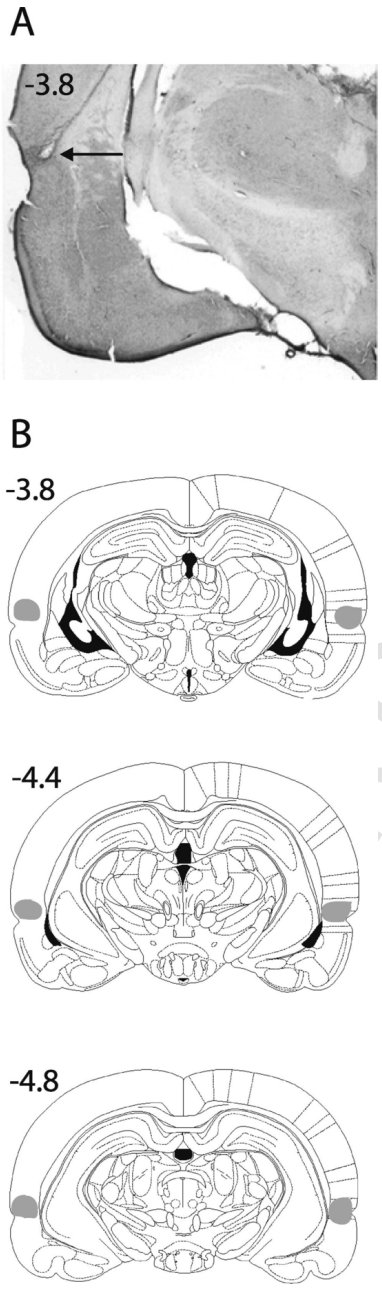

Fig. 4. A) Photomicrograph showing a very small excitotoxic lesion (arrow) around the tip of the infusion cannula, within area 36 of Prh. B) The gray area represents the location within the Prh in which the tips of the infusion needles were identified. AP coordinates are shown in relation to the bregma.

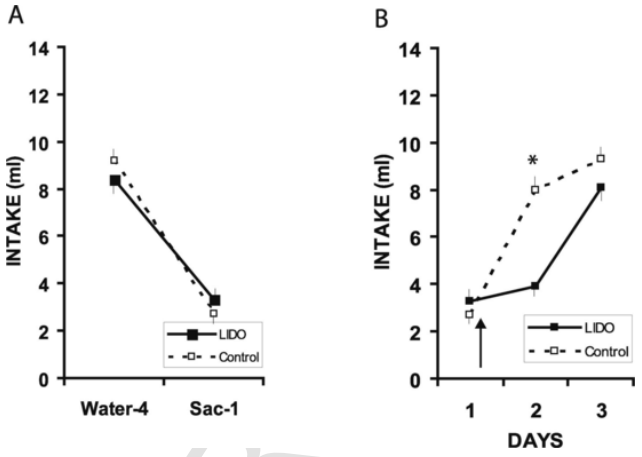

Fig. 5. A) Experiment 3: Mean (±SEM) water and saccharin intake during the last day of water access and the first taste trial. B) Experiment 3: Mean (±SEM) saccharin intake across the 3 successive taste trials. Arrow indicates when lidocaine was infused. * p < 0.01.

3.4. Experiment 4

3.4.1. Histological results

The location of the tips of the injection cannulas was essentially the same as in the previous experiment, all being limited to area 36 (Fig. 4).

3.4.2. Behavioral results

The main results are depicted in Fig. 6A and 6B. Experimental and control groups showed a robust neophobic response the first time they were presented with saccharin. Thus, a significant reduction in intake of the novel saccharin was observed in the first neophobia trial as compared to the last day of baseline water intake ($F_{1, 11}$ group = 0.30, p = 0.59, η^2_p = 0.02; $F_{1, 11}$ trial = 89.35, p < 0.0001, η^2_p = 0.89; $F_{1, 11}$ group × trial = 0.01, p = 0.92, η^2_p = 0.01). Additionally, upon analyzing the intake of saccharin over the course of the six neophobia trials, a 2-way mixed ANOVA revealed a significant effect in the

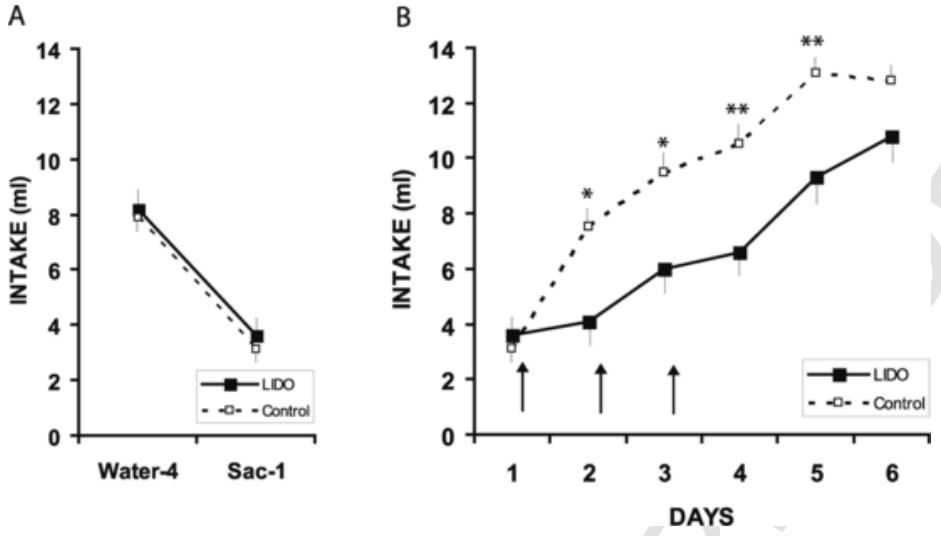

Fig. 6. A) Experiment 4: Mean (±SEM) water and saccharin intake during the last day of water access and the first taste trial. B) Experiment 4: Mean (±SEM) saccharin intake across the 6 successive taste trials. Arrows indicate when lidocaine was infused. ** $p < 0.01$, * $p < 0.05$.

group factor, trial factor and group × trial interaction ($F_{1, 11}$ group = 15.99, $p < 0.01$, $\eta^2_p = 0.59$; $F_{5, 55}$ trial = 101.19, $p < 0.0001$, $\eta^2_p = 0.90$; $F_{5, 55}$ group × trial = 6.03, $p < 0.001$, $\eta^2_p = 0.35$). Tukey tests for the analysis of the interaction showed significant differences between controls and lidocaine-injected rats over all the days except in trial 1 and trial 6 (trial 1, $p = 0.99$; trial 2, $p < 0.02$; trial 3, $p < 0.05$; trial 4, $p < 0.01$; trial 5, $p < 0.01$; trial 6, $p = 0.29$). To better understand the attenuation of the neophobia, we analyzed the saccharin intake of each group over the days. In the control group, the intake in trial 2 was more than double that of trial 1 ($p < 0.0001$). No significant differences were detected, however, when comparing day 2 to day 3 ($p = 0.34$) nor day 3 to day 4 ($p = 0.58$). Finally, a significant increment was observed between day 4 and day 5 ($p < 0.01$), with asymptote being reached on day 6 (trial 5 vs 6, $p = 0.99$). This pattern contrasts with the performance of the Prh lidocaine-injected group that consumed almost the same amount of saccharin in trial 2 as in trial 1 ($p = 0.98$). On the rest of the days that the rats were injected with lidocaine immediately after saccharin intake, Tukey tests indicated no significant differences between day 2 and day 3 ($p = 0.19$) nor between day 3 and day 4 ($p = 0.99$). Importantly, when the experimental rats stopped receiving the Prh lidocaine infusion, the intake of saccharin increased sharply (trial 4 vs 5, $p < 0.01$). Upon comparing trial 4 and trial 5, Cohen's d effect size was 0.63. Finally, no differences were observed upon comparing trial 5 to trial 6 ($p = 0.89$).

It is important to note that the maximum increment in saccharin intake in the experimental group occurred once the three microinjections with lidocaine stopped, that is, from trial 4 to 5. This suggests that the temporary silencing of Prh interferes with the plastic changes necessary for a novel taste to become familiar and safe. Alternatively, if the lidocaine microinfusions were producing taste aversion learning, there would not have been such an increment in saccharin intake after this massive CS-US pairing. However, Cohen's d effect size corresponding to the increase in saccharin intake just after the lidocaine infusions ended was similar in the experimental groups of expt. 3 and 4 (0.68 and 0.63, respectively), suggesting that lidocaine does not cause aversive effects. Therefore, overall, the data from experiment 3 and 4 suggest that Prh plays a certain role in the attenuation of neophobia.

4. Discussion

Animals must choose what they eat carefully, giving priority to safe and familiar foods, in order to ensure their survival. To do this central mechanisms involved in gustatory processing must interact with memory systems to determine whether a food is novel or familiar and, in the event that it has been consumed before, remember what the post-ingestive consequences were. In this study we investigated the effect of lesions to the Prh, a key region in the recognition memory system (Brown & Banks, 2015; Brown et al., 2010), in taste neophobia and its attenuation. In experiments 1a-1c, which used a two-bottle choice test (saccharin vs water), the main data indicated that permanent excitotoxic lesions to the Prh disrupted the neophobic response to 0.3% and 0.5% saccharin in trial 1. On the other hand, Prh lesions did not impair neophobia attenuation and in trials 2–4 lesioned and control subjects drank similar amounts of saccharin, reaching asymptote intake in trial 4. A normal and robust neophobic response, however, was found in Prh-lesioned rats given a 0.7% saccharin solution. A second set of data indicate that when the same animals used in experiments 1a-1c were subjected to a flavor preference learning on the basis of a flavor-taste association, lesioned and control rats acquired the task perfectly, showing during a choice test a clear preference for the flavor associated with the saccharin (experiments 2a-2c). Finally, a third set of experiments, using lidocaine inactivation of the Prh immediately after saccharin intake, indicated that neural activity within this region is necessary for attenuation of neophobia (expts. 3 and 4).

The present findings support and broaden our previous conclusions that Prh is essential for the occurrence of taste neophobia (Ramos, 2015), but now with a two-bottle choice test. One possible interpretation of the trial 1 performance observed in experiments 1a and 1b is that Prh lesions caused a deficit in taste processing/perception and rats treat the saccharin solution as if it were more diluted. Two points, however, do not support this interpretation. First, if Prh lesions had caused a reduction in perceived stimulus intensity, lesioned rats would not have shown such a robust neophobia when presented with saccharin at 0.7%. However, in this case experimental and control groups behave similarly. A possible explanation for this last finding is that in addition to a "sweet" component, saccharin contains a "bitter" component (Horne, Lawless, Speirs, & Sposato, 2002; Kuhn et al., 2004). Thus, a more concentrated saccharin solution (saccharin at 0.7%, for example) may be perceived by the animals as qualitatively aversive, and is normally a non-preferred solution (Smith & Sclafani, 2002; Strouthes, 1977; Wong, 1985). The important point here is that Prh-lesioned animals of expt. 1c are sensitive to this qualitative modification of the stimulus, reducing intake of it to the same degree as the control animals did, which suggests intact taste processing. Indeed, lesioned and control subjects of expt. 1c present a similar saccharin preference index in trial 1, consuming 4-5 times more water than saccharin. Thus, Prh-lesioned rats are perfectly capable of detecting a gusta-

tory stimulus and rejecting it, particularly when it is qualitatively aversive. In support of the foregoing, a similar result has been obtained recently using a 3% cider vinegar solution (Morillas, Gómez-Chacón, & Gallo, 2017). Since vinegar has a sour taste and contains various acidic elements it is avoided by rodents and can, like high concentrations of saccharin, produce affectively negative reactions (Schier & Spector, 2019). In congruence with our results in expt. 1c, the authors of the aforementioned experiment found a similar level of neophobic response in trial 1 in Prh-lesioned and sham rats (Morillas et al., 2017). A similar effect has also been observed following excitotoxic lesions to the lateral parabrachial nucleus. Specifically, parabrachial lesions eliminated the neophobic response to appetitive sapid stimuli (0.15% saccharin and 0.3 M alanine), but had no influence when presented with aversive gustatory solutions (0.0001 M quinine) or aversive olfactory stimuli such as almond odor (Reilly & Trifunovic, 2001). Second, if Prh lesions had caused a reduction in perceived stimulus intensity or in taste processing in general, lesioned animals would present a certain deficit in experiments 2a-2c, yet Prh rats discriminated just as well as controls between the two unsweetened flavors (CS+ vs CS−) in the choice test.

Relative to the data observed in the flavor-taste preference learning experiments (expts. 2a-2c), it is well known that the amygdala is necessary in this type of learning. So, rats with large amygdala lesions did not acquire a preference for a flavor paired with the sweet taste of fructose (Dwyer, 2011; Touzani & Sclafani, 2005). In addition, the infusion of dopamine D_1-like (SCH23390) and D_2-like (raclopride) antagonists into the amygdala impaired the acquisition of flavor-taste learning induced by fructose/saccharin (Bernal et al., 2009). These data are important, firstly, because Prh and the amygdala are anatomically adjacent regions and, secondly, because some studies have shown a deficit in taste neophobia in amygdala-lesioned rats (Dunn & Everitt, 1988; Reilly, 2018b). In consequence, it could be argued that the deficits in taste neophobia observed in experiments 1a and 1b, could be the result of the Prh lesions extending to the amygdala. However, the fact that our Prh-lesioned rats present an intact flavor-taste preference learning rules out this possibility and supports a direct contribution of the Prh in taste neophobia.

An interesting question that deserves certain discussion is how the Prh contributes to taste neophobia. Data from expts. 1a and 1b suggest that lesioned animals treat the novel taste as if it were safer and more familiar than it really is. This implies that lesioned subjects are incapable of evaluating the potential danger associated with any new stimulus, and consequently an increase in the intake of the novel taste is observed in trial 1. A number of studies support this possibility. First, electrophysiological recordings from single neurons in monkeys and rats have shown in Prh a higher proportion of neurons that respond more strongly to novel than to familiar visual stimuli (Xiang & Brown, 1998; Zhu et al., 1995). This same effect, known as "repetition suppression", has been observed in rats when using gustatory stimuli. Specifically, intake of a novel flavor solution induced a higher number of Prh c-Fos-positive neurons in trial 1 than in trial 2 or in trial 6 (Gómez-Chacón et al., 2015). Likewise, using immunohistochemical imaging techniques in rats, other studies have shown that the presentation of novel stimuli, as compared to familiar, produced a greater level of activation of the Prh (Albasser et al., 2010; Wan et al., 1999). Secondly, behavioral studies indicate that Prh-lesioned rats are impaired in the performance of object recognition tasks in which the animals must discriminate between the novelty/familiarity of the stimuli used (Brown, 2009; Mumby & Pinel, 1994; Winters et al., 2008). Third, some authors have observed an attenuated acquisition of CTA after Prh (Gutiérrez, De la Cruz, Rodríguez-Ortiz, & Bermúdez-Rattoni, 2004; Tassoni, Lorenzini, Baldi, Sacchetti, & Bucherelli, 2000), insular cortex (Roman & Reilly, 2007; Roman et al., 2009; see also, Lin, Arthurs, & Reilly, 2011) and basolateral amygdala lesions (St. Andre & Reilly, 2007). Since these lesions also produce a disruption of taste neophobia (Lin et al., 2018; Ramos, 2015), it has been proposed that the CTA deficit could be secondary to the neophobic deficit (Reilly, 2018b). This implies that in a paradigm using CTA, insular cortex, basolateral amygdala and, in our case, Prh-lesioned rats, they would treat the novel stimulus (CS) as if it were familiar, which causes a delay in CTA due to a latent inhibition-like effect (Reilly, 2018b). Supporting this hypothesis, some authors have shown that rats with excitotoxic lesions to the insular cortex acquired a CTA using a novel taste as CS at the same slow rate as the control subjects when a familiar taste was employed as CS (Roman et al., 2009). To further examine this possibility, future studies should investigate whether Prh-lesioned rats acquire a CTA with similar characteristics as observed in insular-lesioned rats.

To investigate whether Prh is necessary for a novel taste to become safe and familiar, we blocked the neural activity of this region by infusing it with lidocaine immediately after the intake of saccharin in trial 1 (expt. 3) and in trials 1, 2 and 3 (expt. 4). In experiment 3, lidocaine completely blocked the attenuation of the neophobia and experimental rats drank in trial 2 a volume of saccharin similar to the one registered in trial 1. However, without the effect of the lidocaine the intake increased abruptly when comparing trial 2 vs trial 3, indicating a normal attenuation of the neophobia. A potential interpretation of the reduction of saccharin intake in trial 2 is that lidocaine infused into the Prh may somehow have functioned as a US, favoring the development of a mild CTA. This hypothesis was tested in a follow-up experiment in which lidocaine was infused into the Prh during three consecutive trials. The rationale was that if lidocaine infusion functions as an US, then three CS-US trials would increase the strength of the aversion in comparison to a single CS-US trial (Riley & Mastropaolo, 1989; see also, Roman & Reilly, 2007). This implies that the experimental rats of expt. 4 should present a greater reduction in the intake of saccharin than the experimental rats of expt. 3, once the infusions ended. However, the results of expt. 4 did not confirm this. Prh lidocaine inactivation prevented the neophobia attenuation from developing normally during the three days of infusion; however, once the infusions ended the intake of saccharin increased significantly (trial 4 vs 5). Importantly, upon comparing the performance of experimental rats from expt. 3 and expt. 4, a greater aversion is not appreciated in infused rats with 3 consecutive CS-US trials compared to those with a single CS-US trial. In consequence, these data encourage the view that Prh lidocaine inactivation disrupted the attenuation of the neophobia without affecting the development of CTA. This conclusion agrees with a previous paper that showed that infusions of lidocaine in the parabrachial nucleus did not produce CTA (i.e., did not serve as a US). Specifically, rats receiving sequential infusions of lidocaine and anisomycin in the parabrachial region presented less taste aversion than rats receiving only the infusion of anisomycin (Lin, Amodeo, Arthurs, & Reilly, 2012). Our results are also in line with a previous paper indicating that Prh-cholinergic activity is critical in neophobia attenuation (Gutiérrez et al., 2004).

Finally, in relation to neophobia attenuation, a previous study found that Prh excitotoxic lesions disrupted the attenuation of the neophobia but not the initial neophobic response (Morillas et al., 2017). As discussed above, it is conceivable that Prh lesions do not interrupt the initial neophobic response to certain gustatory stimuli (see expt. 1c), but the reason that such lesions delay the attentuation in the preceding study is less evident. The authors themselves indicate that the piriform cortex was damaged in some rats, so it may be that extraperirhinal damage contributed to this effect. A recent study by the same group supports this possibility. Specifically, the authors showed that the number of c-Fos positive cells in the posterior piriform cortex in adult rats was higher after the presentation of the taste over a period of 6 days, in comparison to the first or second day of presentation (Grau-Perales, Gómez-Chacón, Morillas, & Gallo, 2019). Thus, in harmony with the foregoing, damage to the piriform region is compatible with a slower attenuation of taste neophobia. Alternatively, as the authors point out, it may be that the Prh lesion has a greater impact

on neophobia attentuation when a taste with a strong odor component is used, such as vinegar (Morillas et al., 2017, p. 233).

To conclude, the Prh has been clearly seen to be involved in the discrimination between novel and familiar items and in related memory processes. Using different lesion methods, we have affected, in the same structure, two different processes related to gustatory neophobia. Permanent excitotoxic lesions to the Prh disrupted the initial neophobic response; however, Prh lidocaine inactivation after the first experience with the novel taste prevented this taste from becoming familiar and safe. Future investigation should focus on determining how the Prh interacts with the rest of the structures involved in gustatory neophobia to carry out each of these two functions.

CRediT authorship contribution statement

Juan M.J. Ramos: Conceptualization, Methodology, Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2020.107264.

References

Agster, K L, & Burwell, R D (2009). Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Hippocampus, 19, 1159–1186.

Agster, K L, Pereira, I T, Saddoris, M P, & Burwell, R D (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. II. Efferents. Hippocampus, 26, 1213–1230.

Ahn, J-R, Lee, H-W, & Lee, I (2019). Rhythmic pruning of perceptual noise for object representation in the hippocampus and perirhinal cortex in rats. Cell Reports, 26, 2362–2376.

Albasser, M M, Poirier, G L, & Aggleton, J P (2010). Qualitatively different modes of perirhinal-hippocampal engagement when rats explore novel vs. familiar objects as revealed by C-Fos imaging. European Journal of Neuroscience, 31, 134–147.

Arthurs, J, Lin, J-Y, & Reilly, S (2018). Inhibiting gustatory thalamus or medial amygdala has opposing effects on taste neophobia. Neurobiology of Learning and Memory, 156, 24–32.

Arthurs, J, & Reilly, S (2013). Role of the gustatory thalamus in taste learning. Behavioural Brain Research, 250, 9–17.

Bahar, A, Dorfman, N, & Dudai, Y (2004). Amygdalar circuits required for either consolidation or extinction of taste aversion memory are not required for reconsolidation. European Journal of Neuroscience, 19, 1115–1118.

Bermúdez-Rattoni, F (2004). Molecular mechanisms of taste-recognition memory. Nature Reviews Neuroscience, 5, 209–217.

Bernal, S, Miner, P, Abayev, Y, Kandova, E, Gerges, M, Touzani, K, … Bodnar, R J (2009). Role of amygdala dopamine D_1 and D_2 receptors in the acquisition and expression of fructose-conditioned flavor preferences in rats. Behavioural Brain Research, 205, 183–190.

Bernstein, I L (1999). Taste aversion learning: A contemporary perspective. Nutrition, 15, 229–234.

Best, M R, Domjan, M, & Hanskins, W L (1978). Long-term retention of flavor familiarization: Effects of number and amount of prior exposures. Behavioral Biology, 23, 95–99.

Brown, M W (2000). Neuronal correlates of recognition memory. In Bolhuis, J J (Ed.), Brain, Perception and Memory (pp. 185–208). Oxford: Oxford University Press.

Brown, M W (2009). Recognition memory: What's new in novelty signals? Current Biology, 19, R645-647.

Brown, M W, & Banks, P J (2015). In search of a recognition memory engram. Neuroscience and Biobehavioral Reviews, 50, 12–28.

Brown, M W, Warburton, E C, & Aggleton, J P (2010). Recognition memory: Material, processes, and substrates. Hippocampus, 20, 1228–1244.

Bures, J, Bermúdez-Rattoni, F, & Yamamoto, T (1998). Conditioned taste aversion: Memory of a special kind. Oxford, UK: Oxford University Press.

Burwell, R D (2001). Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. Journal of Comparative Neurology, 437, 17–41.

Burwell, R D, & Amaral, D G (1998). Cortical afferents of the perirhinal cortex, postrhinal, and entorhinal cortices of the rat. Journal of Comparative Neurology, 398, 1–27.

Burwell, R D, Witter, M P, & Amaral, D G (1995). Perirhinal and postrhinal cortices of the rat: A review of the neuroanatomical literature and comparison with findings from the monkey brain. Hippocampus, 5, 390–408.

Bussey, T J, Muir, J L, & Aggleton, J P (1999). Functionally dissociating aspects of event memory: The effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. Journal of Neuroscience, 19, 495–502.

Cechetto, D F, & Saper, C B (1987). Evidence for a viscerotopic sensory representation in the cortex and the thalamus in the rat. Journal of Comparative Neurology, 262, 27–45.

Corey, D T (1978). The determinants of exploration and neophobia. Neuroscience and Biobehavioral Reviews, 2, 235–253.

Díaz, E, & De la Casa, L G (2011). Extinction, spontaneous recovery and renewal of flavor preferences based on taste-taste learning. Learning and Motivation, 42, 64–75.

Díaz, E, De la Casa, L G, Ruiz, G, & Baeyens, F (2004). Taste-taste learning on the acquisition of the flavor preferences. Psicológica, 25, 135–146.

Domjan, M (1976). Determinants of the enhancement of the flavored-water intake by prior exposure. Journal of Experimental Psychology: Animal Behavior Processes, 2, 17–27.

Dunn, L T, & Everitt, B J (1988). Double dissociation of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. Behavioral Neuroscience, 102, 3–23.

Dwyer, D M (2011). Lesions of the basolateral, but not central, amygdala impair flavour-taste learning based on fructose o quinine reinforcers. Behavioural Brain Research, 220, 349–353.

Eichenbaum, H, Yonelinas, A P, & Ranganath, C (2007). The medial temporal lobe and recognition memory. Annual Review of Neuroscience, 30, 123–152.

Elizalde, G, & Sclafani, A (1990). Flavor preferences conditioned by intragastric Polycose: A detailed analysis using an electronic esophagus preparation. Physiology & Behavior, 47, 63–77.

Ennaceur, A, Neave, N, & Aggleton, J P (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. Behavioural Brain Research, 80, 9–25.

Fahy, F L, Riches, I P, & Brown, M W (1993). Neuronal activity related to visual recognition memory: Long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. Experimental Brain Research, 96, 457–472.

Fanselow, M S, & Birk, J (1982). Flavor-flavor associations induce hedonic shifts in taste preference. Animal Learning & Behavior, 10, 223–228.

Figueroa-Guzmán, Y, & Reilly, S (2008). NMDA receptors in the basolateral amygdala and gustatory neophobia. Brain Research, 1210, 200–203.

Forestell, C A, & Lolordo, V M (2003). Palatability shifts in taste and flavour preference conditioning. The Quarterly Journal of Experimental Psychology, 56B, 140–160.

Furtak, S C, We, S-M, Agster, K L, & Burwell, R D (2007). Functional neuroanatomy of the parahippocampal region in the rat: The perirhinal and postrhinal cortices. Hippocampus, 17, 709–722.

García, J, Kimmeldorf, D J, & Koelling, R A (1955). Conditioned taste aversion to saccharin resulting from exposure to gamma radiation. Science, 122, 157–158.

Gilbert, P E, Campbell, A, & Kesner, R P (2003). The role of the amygdala in conditioned flavor preference. Neurobiology of Learning and Memory, 79, 118–121.

Gómez-Chacón, B, Morillas, E, & Gallo, M (2015). Altered perirhinal cortex activity patterns during taste neophobia and their habituation in aged rats. Behavioural Brain Research, 281, 245–249.

Grau-Perales, A, Gómez-Chacón, B, Morillas, E, & Gallo, M (2019). Flavor recognition memory related activity of the posterior piriform cortex in adult and aged rats. Behavioural Brain Research, 360, 196–201.

Gutiérrez, R, De la Cruz, V, Rodríguez-Ortiz, C J, & Bermúdez-Rattoni, F (2004). Perirhinal cortex muscarinic receptor blockade impairs taste recognition memory formation. Learning & Memory, 11, 95–101.

Gutiérrez, R, Téllez, L A, & Bermúdez-Rattoni, F (2003). Blockade of cortical muscarinic but not NMDA receptors prevents a novel taste from becoming familiar. European Journal of Neuroscience, 17, 1556–1562.

Ho, J W, Poeta, D L, Jacobson, T K, Zolnik, T A, Neske, G T, Connors, B W, & Burwell, R D (2015). Bidirectional modulationn of recognition memory. Journal of Neuroscience, 35, 13323–13335.

Holman, E W (1975). Inmediate and delayed reinforcers for flavor preferences in rats. Learning and Motivation, 6, 91–100.

Hölscher, C, Rolls, E T, & Xiang, J (2003). Perirhinal cortex neuronal activity related to long-term familiarity in the macaque. European Journal of Neuroscience, 18, 2037–2046.

Horne, J, Lawless, H T, Speirs, W, & Sposato, D (2002). Bitter taste of saccharin and acesulfame-K. Chemical Senses, 27, 31–38.

Kosar, E, Grill, H J, & Norgren, R (1986). Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. Brain Research, 379, 329–341.

Kuhn, C, Bufe, B, Winning, M, Hofmann, T, Frank, O, Behrens, M, … Meyerhof, W (2004). Bitter taste receptors for saccharin and acesulfame K. Journal of Neuroscience, 24, 10260–10265.

Li, L, Miller, E K, & Desimone, R (1993). The representation of stimulus familiarity in anterior inferior temporal cortex. Journal of Neurophysiology, 69, 1918–1929.

Lin, J-Y, Amodeo, L R, Arthurs, J, & Reilly, S (2012). Anisomycin infusions in the parabrachial nucleus and taste neophobia. Neurobiology of Learning and Memory, 98, 348–353.

Lin, J-Y, Arthurs, J, & Reilly, S (2011). Role of the insular cortex in morphine-induced conditioned taste avoidance. Brain Research, 1384, 80–88.
Lin, J-Y, Arthurs, J, & Reilly, S (2018). The effects of amygdala and cortical inactivation on taste neophobia. Neurobiology of Learning and Memory, 155, 322–329.
Lin, J-Y, & Reilly, A (2012). Amygdala-gustatory insular cortex connections and taste neophobia. Behavioural Brain Research, 235, 182–188.
Lin, J-Y, Roman, C, Arthurs, J, & Reilly, S (2012). Taste neophobia and c-Fos expression in the rat brain. Brain Research, 1448, 82–88.
Lin, J-Y, Roman, C C, St. Andre, J, & Reilly, S (2009). Taste, olfactory and trigeminal neophobia in rats with forebrain lesions. Brain Research, 1251, 195–203.
Martin, J H (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neuroscience Letters, 127, 160–164.
Messier, C, & White, N M (1984). Contingent and non-contingent actions of sucrose and saccharin reinforcers: Effects on taste preference and memory. Physiology & Behavior, 32, 195–203.
Milgram, N W, Krames, L, & Alloway, T M (Eds.). (1977). Food Aversion Learning. New York: Plenum Press.
Miller, R R, & Holzman, A D (1981). Neophobia: Generality and function. Behavioral and Neural Biology, 33, 17–44.
Monk, K J, Rubin, B D, Keene, J C, & Katz, D B (2014). Licking microstructure reveals rapid attenuation of neophobia. Chemical Senses, 39, 203–213.
Moraga-Amaro, R, Cortés-Rojas, A, Simon, F, & Stehberg, J (2014). Role of the insular cortex in taste familiarity. Neurobiology of Learning and Memory, 109, 37–45.
Morillas, E, Gómez-Chacón, B, & Gallo, M (2017). Flavor and object recognition memory impairment induced by excitotoxic lesions of the perirhinal cortex. Neurobiology of Learning and Memory, 144, 230–234.
Mumby, D G, & Pinel, J P J (1994). Rhinal cortex lesions and object recognition in rat. Behavioral Neuroscience, 108, 11–18.
Nachman, M, & Ashe, J H (1974). Effects of basolateral amygdala lesions on neophobia, learned taste aversion, and sodium appetite in rats. Journal of Comparative and Physiological Psychology, 87, 622–643.
Osorio-Gómez, D, Guzmán-Ramos, K, & Bermúdez-Rattoni, F (2018). Neurobiology of neophobia and its attenuation. In Reilly, S (Ed.), Food neophobia: Behavioral and biological influences (pp. 111–128). Elsevier.
Otto, T, & Eichenbaum, H (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. Behavioral Neuroscience, 106, 762–775.
Paxinos, G, & Watson, C (1998). The rat brain in stereotaxic coordinates (4th ed.). New York: Academic Press.
Pereira, I T, Agster, K L, & Burwell, R D (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. I. Afferents. Hippocampus, 26, 1189–1212.
Pereira de Vasconcelos, A, Klur, S, Muller, C, Cosquer, B, López, J, Certa, U, & Cassel, J C (2006). Reversible inactivation of the dorsal hippocampus by tetrodotoxin or lidocaine: A comparative study on cerebral functional activity and motor coordination in the rat. Neuroscience, 141, 1649–1663.
Ramos, J M J (2015). Differential contribution of perirhinal cortex and hippocampus to taste neophobia: Effect of neurotoxic lesions. Behavioural Brain Research, 284, 94–102.
Reilly, S (2018). Taste neophobia: Neural substrates and palatability. In Reilly, S (Ed.), Food neophobia: Behavioral and biological influences (pp. 77–109). Elsevier.
Reilly, S (Ed.). (2018). Food neophobia: Behavioral and biological influences. Elsevier.
Reilly, S, & Pritchard, T C (1996). Gustatory thalamus lesions in the rat: II. Aversive and appetitive taste conditioning. Behavioral Neuroscience, 110, 746–759.
Reilly, S, & Schachtman, T R (Eds.). (2009). Conditioned taste aversion: Behavioral and neural processes. New York: Oxford University Press.
Reilly, S, & Trifunovic, R (2001). Lateral parabrachial nucleus lesions in the rat: Neophobia and conditioned taste aversión. Brain Research Bulletin, 55, 359–366.
Renteria, A F, Silbaugh, B C, Tolentino, J C, & Gilbert, P E (2008). Age-related changes in conditioned flavor preference in rats. Behavioural Brain Research, 188, 56–61.
Riley, A L, & Mastropaolo, J P (1989). Long-delay taste aversion learning: Effects of repeated trials and two-bottle testing conditions. Bulletin of the Psychonomic Society, 27, 145–148.
Roman, C, Lin, J-Y, & Reilly, S (2009). Conditioned taste aversion and latent inhibition following extensive taste preexposure in rats with insular cortex lesions. Brain Research, 1259, 68–73.
Roman, C, & Reilly, S (2007). Effects of insular cortex lesions on conditioned taste aversion and latent inhibition in the rat. European Journal of Neuroscience, 26, 2627–2632.
Rozin, P, & Zellner, D (1985). The role of Pavlovian conditioning in the acquisition of food likes and dislikes. Annals of the New York Academy of Sciences, 443, 189–202.
Schier, L A, & Spector, A C (2019). The functional and neurobiological properties of bad taste. Physiological Reviews, 99, 605–663.
Sclafani, A (2004). Oral and postoral determinants of food reward. Physiology & Behavior, 81, 773–779.
Sclafani, A, & Ackroff, K (1994). Glucose-conditioned and fructose-conditioned flavor preferences in rats – taste versus postingestive conditioning. Physiology & Behavior, 56, 399–405.
Shi, C-J, & Cassel, M D (1999). Perirhinal cortex projections to the amygdaloid complex and hippocampal formation in the rat. Journal of Comparative Neurology, 406, 299–328.
Siegel, S (1974). Flavor preexposure and "learned safety". Journal of Comparative and Physiological Psychology, 87, 1073–1082.
Smith, J C, & Sclafani, A (2002). Saccharin as a sugar surrogate revisited. Appetite, 38, 155–160.
St. Andre, J, & Reilly, S (2007). Effects of central and basolateral amygdala lesions on conditioned taste aversion and latent inhibition. Behavioral Neroscience, 121, 90–99.
Stehberg, J, Moraga-Amaro, R, & Simon, F (2011). The role of the insular cortex in taste function. Neurobiology of Learning and Memory, 96, 130–135.
Strouthes, A (1977). Saccharin eating in undeprived and hungry rats. Animal Learning & Behavior, 5, 42–46.
Suzuki, W A, & Amaral, D G (1994). Perirhinal and parahippocampal cortices of the macaque monkey: Cortical afferents. Journal of Comparative Neurology, 350, 497–533.
Tassoni, G, Lorenzini, C A, Baldi, E, Sacchetti, B, & Bucherelli, C (2000). Role of the perirhinal cortex in rats' conditioned taste aversion response memorization. Behavioral Neuroscience, 114, 875–881.
Tehovnik, E J, & Sommer, M A (1997). Effective spread and time course of neural inactivation caused by lidocaine injection in monkey cerebral cortex. Journal of Neuroscience Methods, 74, 17–26.
Touzani, K, & Sclafani, A (2005). Critical role of amygdala in flavor but not taste preference learning in rats. European Journal of Neuroscience, 22, 1767–1774.
Ueji, K, Minematsu, Y, Takeshita, D, & Yamamoto, T (2016). Saccharin taste conditions flavor preference in wealing rats. Chemical Senses, 41, 135–141.
von Linstow Roloff, E, Muller, R U, & Brown, M W (2016). Finding and not finding rat perirhinal neuronal responses to novelty. Hippocampus, 26, 1021–1032.
Wan, H, Aggleton, J P, & Brown, M W (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. Journal of Neuroscience, 19, 1142–1148.
Winters, B D, & Bussey, T J (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. Journal of Neuroscience, 25, 52–61.
Winters, B D, Forwood, S E, Cowell, R A, Saksida, L M, & Bussey, T J (2004). Double dissociation between the effects of peri-posthrinal cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. Journal of Neuroscience, 24, 5901–5908.
Winters, B D, Saksida, L M, & Bussey, T J (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. Neuroscience and Biobehavioral Reviews, 32, 1055–1070.
Wong, R (1985). Saccharin preference of hamsters, gerbils and rats in the two-food situation. Behavioural Processes, 10, 87–99.
Xiang, J-Z, & Brown, M W (1998). Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. Neuropharmacology, 37, 657–676.
Yamamoto, T, & Ueji, K (2011). Brain mechanisms of flavor learning. Frontiers in Systems Neuroscience, 5, e00076.
Young, B J, Otto, T, Fox, G D, & Eichenbaum, H (1997). Memory representation within the parahippocampal region. Journal of Neuroscience, 17, 5183–5195.
Zhu, X O, Brown, M W, & Aggleton, J P (1995). Neuronal signalling of information important to visual recognition memory in rat rhinal and neighbouring cortices. European Journal of Neuroscience, 7, 753–765.