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DISCONNECTION OF THE PERIRHINAL AND INSULAR CORTICES SEVERELY DISRUPTS TASTE NEOPHOBIA

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Abstract

It is well known that the perirhinal (Prh) and insular (IC) cortices are reciprocally connected, mainly through ipsilateral projections. Although some studies have demonstrated that excitotoxic lesions to these regions, each separately, disrupt taste neophobia, it is not yet known whether the two regions have functional interactions with one another. To find out if they form a functional unit, we examined the effects of crossed excitotoxic lesions to the Prh and the contralateral IC (contralateral group). This group's performance was compared to that of rats with ipsilateral Prh and IC lesions (ipsilateral group) and to that of control-operated rats. All the animals received a 0.3% saccharin solution for fifteen minutes on five consecutive days. Rats with contralateral Prh-IC lesions drank significantly higher amounts of saccharin than the other groups during the first encounter with the novel taste, indicating a disruption in neophobia. However, the lesions did not disrupt attenuation of neophobia, with the contralateral group reaching asymptote in trial 2 and the rest of the groups after 3-5 days of exposure to the saccharin. These findings suggest that both Prh and IC play a necessary role in taste neophobia. Additionally, the two cortices function interdependently and their interaction is critical for normal expression of taste neophobia.

Keywords: Perirhinal Cortex; Insular Cortex; Medial Temporal Lobe; Neophobia; Taste Learning

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1. Introduction

Typically, when rats and other animals come across new foods in their habitat, they are reluctant to consume them and will eat only a small amount. This phenomenon is termed taste neophobia and it serves to limit the intake of new foods until the subject knows what their post-ingestive consequences are. Taste neophobia thus works as a defense mechanism, increasing the chances of the animal's survival by preventing the over-consumption of potentially toxic new foods. If the ingestion of the new tastant is not followed by aversive post-ingestive consequences, the new taste will gradually be deemed safe and thus more pleasurable, and intake will increase in subsequent encounters with it, a phenomenon known as attenuation of neophobia (Bermúdez-Rattoni, 2004; Lin, Amodeo, Arthurs, & Reilly, 2012; Reilly, 2018a). On the other hand, if intake of the novel tastant results in gastrointestinal illness, rats will develop conditioned taste aversion (CTA) and show decreased intake the next time they encounter it (Bures, Bermúdez-Rattoni, & Yamamoto, 1998).

The brain mechanisms underlying taste neophobia are not yet well understood. Recent studies have identified some structures that are clearly implicated, such as basolateral amygdala, gustatory insular cortex, gustatory thalamus and medial amygdala (for review see Reilly, 2018b; Osorio-Gómez, Guzmán-Ramos, & Bermúdez-Rattoni, 2018). A common effect observed after excitotoxic lesions to the above structures is an elevated intake of the novel taste at the initial encounter and an intake similar to that of controls at asymptote after presentation of the taste over several successive days (Lin, Roman, St. Andre, & Reilly, 2009; Arthurs & Reilly, 2013). In congruence with the preceding data, three of the aforementioned regions (basolateral amygdala, gustatory thalamus and gustatory insular cortex) showed more c-Fos immunoreactivity to the taste of a novel saccharin solution than to the familiar saccharin (Lin, Roman, Arthurs, & Reilly, 2012). Finally, the temporary inactivation of any of these structures (by infusion of baclofen/muscimol) before exposure to a novel saccharin solution caused a severe disruption of taste neophobia in trial 1 and, moreover, a deficit in attenuation of

neophobia in trial 2, which suggests implication in both processes (Lin, Arthurs, & Reilly, 2018; Arthurs, Lin, & Reilly, 2018).

Recent research in our laboratory has also implicated the perirhinal cortex (Prh) in taste neophobia. Similar to the data obtained by Reilly and associates, excitotoxic lesions to Prh severely disrupted taste neophobia and lesioned rats drank more novel 0.3% and 0.5% saccharin solution than control animals in trial 1, although the amount of fluid intake at asymptote was equivalent in the two groups. This effect has been observed in two different situations; specifically, using a one-bottle procedure (Ramos, 2015) and a two-bottle procedure (water vs. saccharin, Ramos, 2020) with comparable results and conclusions. In congruence with our results, other authors, using c-Fos immunoreactivity, have shown that the intake of a novel flavor solution produced a higher number of Prh c-Fos-positive neurons during trial 1 than during trials 2 and 6, suggesting that Prh plays a role in taste neophobia (Gómez-Chacón, Morillas, & Gallo, 2015).

The above structures are anatomically connected to each other and taste neophobia depends on there being a precise functional interaction between them (Krettek & Price, 1977; Shi & Cassell, 1998; Lin & Reilly, 2012; Pereira, Agster, & Burwell, 2016; Agster, Pereira, Saddoris. & Burwell, 2016; Furtak, Wei, Agster, & Burwell, 2007). With respect to Prh, several studies have described strong and reciprocal direct connections with the insular cortex, showing dense connections ipsilaterally (Burwell & Amaral, 1998; Agster & Burwell, 2009) but few or minimal connections contralaterally (Deacon, Eichenbaum, Rosenberg, & Eckmann, 1983; Saper, 1982; Reep & Winans, 1982; McIntyre, Kelly, & Staines, 1996). Thus, direct communication between the perirhinal and insular cortices occurs predominantly within the same hemisphere.

Based on the foregoing, the objective of this study was to investigate whether a functional interaction exists between Prh and the insular cortex (IC) and if said interaction is necessary for taste neophobia to occur normally. With this aim we examined the effect of crossed excitotoxic lesions to Prh and IC (contralateral group). The taste neophobia of rats with crossed-

disconnection lesions was compared to that of rats with Prh plus IC lesions in the same hemisphere (ipsilateral group) and to that of sham-operated rats. The rationale behind this was that if perirhinal and insular cortices interact functionally with each other during taste neophobia, then crossed lesions (e.g. Prh lesions in one hemisphere and IC in the other) would be expected to produce a taste neophobia disruption comparable to that observed after bilateral lesions of each structure separately. On the other hand, normal taste neophobia would be expected after Prh-insular cortices lesions in the same hemisphere. Results indicated that the Prh and IC form a functional unit and that the integrity of these two structures is necessary for normal occurrence of taste neophobia.

2. Material and methods

2.1. Subjects

The subjects were 32 male Wistar rats from Charles River Laboratories. The rats, initially weighing between 280-300 g, were individually housed in single polycarbonate cages (480 x 265 x 210 mm, Tecniplast, Italy) and maintained at a constant temperature of 22±1° C. Rats were given *ad libitum* food and water until the experiment started. Experimental procedures were performed in conformity with European and Spanish legislation (2010/63 EEC and BOE 53/2013, respectively) and were approved by the Ethics Committee for Animal Research of the University of Granada.

2.2. Surgery

Under the effects of sodium pentobarbital anesthesia (65 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 assigned randomly to one of the three following groups, and lesion sides (left or right) were counterbalanced within each group. Twelve rats received contralateral lesions to the Prh and IC (contralateral group), ten received ipsilateral lesions to the Prh and IC (ipsilateral group) and ten rats received sham lesions to the Prh and IC (five rats were sham-lesioned contralaterally and five ipsilaterally). Following the histological analyses one rat from

the contralateral group and three from the ipsilateral group were excluded from the sample. The lesions were produced by the administration of N-methyl-D-aspartic acid (NMDA, Sigma-Aldrich, Madrid, Spain, PBS, pH 7.4, 0.07 M) through a 30-gauge stainless steel cannula inserted in several sites of the brain. 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. The Prh was lesioned in three sites. In this case the cannula was oriented laterally at 26° from the vertical and the coordinates, derived from the atlas of Paxinos & Watson (1998), were as follows: 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. In the IC two lesions were produced, with the following stereotaxic coordinates: AP = +1.2, L = ±5.2, V = -5.0; AP = +1.2, L = ±5.2, V = -4.4. NMDA was administered in a 0.3 µl volume at each site through the cannula, which 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 sham-operated group received identical surgical procedures with one exception, equivalent volumes of phosphate-buffered saline (PBS) were infused into the Prh/IC. After surgery, each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, richterpharma, ag, Austria).

2.3. Behavioral procedure

All behavioral testing occurred in the home cages. After recovering from surgery, animals were placed on a water-restriction schedule that consisted of 15 min of water access in the morning followed 6 h later by a second 15 min of water access in the afternoon. After 4 days of this habituation program, on the fifth experimental day, the first presentation of the novel sodium saccharin solution (0.3%, Sigma-Aldrich, Madrid, Spain) took place. The animals received five presentations of saccharin on 5 consecutive days (from experimental day 5 to day 9). The tastant was always presented in the morning, in calibrated tubes fitted with a rubber

stopper and a steel sipper spout extending 1.5 cm into the home cage. Saccharin intake was measured to the nearest 0.1 ml. Six hours after the conclusion of each neophobia trial all rats had unlimited access to water for 15 min.

2.4. Histology

When the behavioral testing was complete, 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 (40 μm) were cut on a cryostat (Leica CM 1850, Leica Microsystems, Germany) and stained with cresyl violet, a Nissl stain.

To quantify the extension of the Prh damage in each lesioned rat, regions of cell loss and gliosis identified microscopically were plotted on drawings of coronal sections from the Paxinos and Watson atlas (1998). The reconstruction of the lesions was created based on five coronal sections (anteroposterior levels from bregma: -3.3, -4.1, -4.8, -5.6 and -6.3 mm). Each coronal section was digitized and the lesioned area was calculated by a computer program (ImageJ, http://imagej.nih.gov/ij/). The anatomical limits of the Prh were defined in accordance with the works of Burwell (Burwell, 2001). To calculate the extension of the IC lesions, the same procedure was followed, using the following five coronal sections (anteroposterior levels from bregma: +2.20, +1.60, +1.0, +0.20 and -0.30 mm). The anatomical limits of the IC were defined in accordance with the works of Norgren and associates (Kosar, Grill, & Norgren, 1986; see also Cechetto & Saper, 1987). The volume of damage was expressed as a percentage, reflecting the amount of lesioned tissue in relation to the same non-lesioned region located in the opposite hemisphere.

2.5. Data analyses

The intake of saccharin was analysed with a 2-way mixed design analysis of variance (ANOVA) with group as the between-subject and trial as the within-subject variable (3 group x 5 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 (3 x 2). Post-hoc Bonferroni tests were used for the analyses of simple main effects. A Bonferroni correction was used to ensure a family-wise α error rate at 0.01. All the analyses were conducted with Statistica software 10.0 (StatSoft, Tulsa, Oklahoma).

3. Results

3.1. Histology

3.1.1. Prh lesion

Tissue damage was microscopically identified by marked thinning of the cortex, the presence of gliosis or the loss of cell bodies. The lesions affected 75-92% of area 36 and 66-80% of area 35, creating a longitudinal groove on both sides of the rhinal fissure. One-way ANOVA indicated similar lesion size when comparing contralateral vs. ipsilateral groups ($F_{1,16} = 0.056$, $p = 0.81$). The lesions extended anteroposteriorally between -3.30 and -6.30 mm in relation to bregma (Fig. 1A). In some rats the lesions caused minor damage in the zones around the Prh (ventral temporal association cortex, lateral entorhinal cortex, postrhinal cortex and CA1 field of the hippocampus) except in two rats of the ipsilateral group in which the lesion had destroyed a large part of the lateral entorhinal cortex. These two rats were eliminated from the analysis. No rats showed any damage in the amygdala. Overall, including areas 36 and 35, the size of the lesions was between 70-87% of the Prh.

3.1.2. IC lesion

Fig. 1B shows a serial reconstruction of the extension of the lesions of the IC. The lesions were mainly limited to the IC with minor extension into surrounding regions such as the somatosensory cortex, piriform cortex and claustrum. One-way ANOVA indicated similar

lesion size in contralateral and ipsilateral groups ($F_{1,16} = 0.13$, $p = 0.72$). The lesions extended rostrocaudally between +2.30 and -0.5 mm in relation to bregma, although the most lesioned area was between +1.6 and +0.7 mm rostral to bregma. On the dorsoventral plane, the lesions were located just dorsal to the rhinal fissure and they affected almost completely the dysgranular and the ventral zone of the granular subregions of the IC. Including dysgranular, granular and posterior agranular subregions, the size of the overall lesion to the IC was between 66-85%. One animal from the ipsilateral group and one from the contralateral group were excluded from the sample because they presented insufficient lesions (<50%). In short, the total number of rats included in the analyses was 28: 11 rats in the contralateral group, 7 in the ipsilateral group and 10 in the sham-operated group.

3.2. Behavioral results

Water intake during the last two days did not differ between groups ($F_{2,25} = 2.44$, $p = 0.11$) and no differences were observed from day 3 to day 4 ($F_{1,25} = 0.39$, $p = 0.53$), nor in the interaction ($F_{2,25} = 1.17$, $p = 0.32$), suggesting stabilized consumption. Likewise, one-way ANOVA detected no differences when comparing water consumption during the last day of habituation ($F_{2,25} = 2.42$, $p = 0.11$). The main findings suggested that unilateral excitotoxic lesions to Prh in one hemisphere plus unilateral excitotoxic lesions to IC in the contralateral but not ipsilateral hemisphere disrupted taste neophobia. These impressions were supported by the following analyses. First, we examined the intake of saccharin on the five consecutive days that animals received the tastant solution. An initial ANOVA showed no significant differences between the two control subgroups (sham-lesioned contralaterally vs. sham-lesioned ipsilaterally) over the five days that saccharin was presented ($F_{1,18}$ group = 0.67, $p = 0.43$; $F_{4,32}$ trial = 20.11, $p<0.0001$; $F_{4,32}$ interaction = 0.83, $p = 0.51$). These data were thus pooled to form a single sham group. The intake of all groups over the five experimental days appears in Fig. 2A. A two-way mixed ANOVA (3 x 5) indicated a significant effect of group ($F_{2,25} = 8.48$, $p<0.001$, $\eta^2_p = 0.40$), trial ($F_{4,100} = 42.07$, $p<0.0001$, $\eta^2_p = 0.62$) and interaction ($F_{8,100} = 4.20$, $p<0.001$, η^2_p

= 0.25). The analysis of the interaction revealed that the contralateral group consumed larger quantities of saccharin than the ipsilateral ($p<0.01$) and sham ($p<0.0008$) groups in the neophobia test on day 1. On day 2, however, significant differences in saccharin intake were only detected between the contralateral and sham groups ($p<0.003$), but not between contralateral vs. ipsilateral groups ($p = 0.08$). Finally, ipsilateral and sham groups consumed similar amounts of saccharin during neophobia trial 1 and 2 ($p = 1$). During the rest of the days (days 3, 4 and 5), Bonferroni tests revealed no significant differences between groups.

Secondly, to determine whether the attenuation of the neophobia was also disrupted in the contralateral group, we analyzed the saccharin intake of each group over the days. In the three groups the saccharin intake in neophobia trial 2 was significantly higher than it had been on day 1 (contralateral, $p<0.003$; ipsilateral, $p<0.006$; sham, $p<0.001$). However, in no group were significant differences found when comparing the saccharin intake of day 3 with that of day 2 ($p = 1$). Last of all, in ipsilateral ($p<0.01$) and sham groups ($p<0.02$) clear differences were found when comparing day 2 to day 5. In the contralateral group, however, no significant differences were found with this comparison ($p = 1$). No other comparison was significant either. This data suggests that in the contralateral group the attenuation of the neophobia was complete in trial 2, while the ipsilateral and sham groups reached asymptote a few days later, between days 3-5.

Thirdly, we looked to see if the quantity of water consumed during the 15 min afternoon period differed between the groups. We reasoned that in the contralateral group, the deficit in the initial taste neophobia response would cause a reduction in the water consumed in comparison with the ipsilateral and sham groups, which presented a robust neophobic response to saccharin. The results are presented in Fig. 2B. A two-way mixed ANOVA (3 x 5) revealed a significant effect of group ($F_{2,25} = 6.11$, $p<0.01$, $\eta^2_p = 0.32$), trial ($F_{4,100} = 41.15$, $p<0.0001$, $\eta^2_p = 0.62$) and interaction ($F_{8,100} = 3.61$, $p<0.001$, $\eta^2_p = 0.22$). Bonferroni post-hoc tests to analyze interaction found significant differences upon comparing contralateral vs. ipsilateral ($p<0.004$) and contralateral vs. sham groups ($p<0.005$), both on experimental day 2, but no other

comparison turned out to be significant. Within-groups comparisons detected significant differences only in the contralateral group, when comparing the intake of day 1 to that of day 2 ($p<0.004$). Overall, these data indirectly suggest a deficit in taste neophobia in rats with contralateral lesions. Keep in mind that in the contralateral group the deficit in taste neophobia is detected in the first encounter with the tastant in trial 1, when the animals have not yet received the afternoon rehydration sessions. So, the deficit in the initial taste neophobia response observed in the contralateral group is independent of the water consumed in the afternoon. The most reasonable interpretation of the foregoing data is that the overconsumption of saccharin during the morning neophobia tests makes the contralateral rats ingest less water in the afternoon, in comparison with the other groups, which present a robust and intact neophobic response in the morning.

4. Discussion

Several studies have shown that taste neophobia is disrupted by damage to either Prh or IC. However, whether these two structures interact directly in normal expression of taste neophobia is still unknown. The current study examined the functional interaction between the Prh and IC, using a crossed-disconnection approach. With this type of procedure, since Prh-IC have strong ipsilateral connections primarily, asymmetric lesions in the Prh-IC circuit block direct interaction between these two cortices without lesioning either structure bilaterally. Results indicated that rats with contralateral/asymmetric Prh-IC excitotoxic lesions, but not ipsilateral lesioned or sham-operated rats, had severely impaired taste neophobia. Thus, contralateral rats consumed significantly more saccharin during the first trial than the rest of the groups. However, Prh-IC contralateral lesions did not completely eliminate the initial neophobic response, producing instead a partial attenuation of taste neophobia in the first taste trial. This could be explained by the fact that in the contralateral group there are still functionally intact structures in the neurocircuitry underlying taste neophobia, which may compensate for the effect of the lesions, for example, an amygdala-gustatory insular cortex system (Lin & Reilly,

2012), or other structures such as the gustatory thalamus (Arthurs & Reilly, 2013), amygdala (Lin, Arthurs, & Reilly, 2018) or parabrachial nucleus (Reilly & Trifunovic, 2001). Finally, as indicated by the foregoing, the contralateral group did not experience a disruption in the attenuation of the neophobia; quite the contrary, our data suggest a faster attenuation. In effect, given the overconsumption of saccharin observed in the contralateral group in trial 1, it is not surprising that this group reached complete attenuation of the neophobia in trial 2, while the rest of the groups reached it some days later (3-5 days). These results suggest, first of all, that Prh and IC are both necessary and, second, that the two cortices function interdependently, their interaction being critical for taste neophobia to occur normally. It could be argued that the deficit observed after Prh-IC crossed lesions is due to a summatory effect of the unilateral lesions in each cortex. However, the same lesions are obviously present in the animals of the ipsilateral group in which no deficit is found. In the ipsilateral group, since the lesions are confined to the same hemisphere, Prh and IC cortices are still able to interact unilaterally and organize an integrated neophobic response. So, the critical difference between the ipsilateral and contralateral group is that in the latter the interaction between the two cortices has been completely abolished by the crossed lesions. This leads us to believe that Prh and IC rely on each other during taste neophobia and are components of the same functional circuit.

Regarding the nature of the deficit caused by the disconnection, it is unlikely that it can be explained by a failure in taste perception/discrimination. First, as regards the IC, some studies have observed that extensive lesions to this region do not produce perceptive deficits (Kiefer & Orr, 1992) nor do they alter discrimination between preferred (sucrose) and non-preferred (quinine) tastes (Stehberg & Simon, 2011). Other studies find a mild, yet significant, disruption of CTA acquisition in IC lesioned rats when 0.1% saccharin is used as a CS, but no deficit whatsoever is observed when 0.5% saccharin is used (Stehberg, Moraga-Amaro, & Simon, 2011). Similarly, when neurologically intact rats acquire a CTA and a few days later the IC is lesioned, the rats become twice as amnesic when 0.1% saccharin is used as CS, as compared to when 0.5% saccharin is used (Stehberg, Moraga-Amaro, & Simon, 2011). Second, in relation to

the Prh, a recent study in our lab indicated that excitotoxic lesions of this region disrupted taste neophobia to novel saccharin at a concentration of 0.3% and 0.5%, but not to saccharin at a concentration of 0.7% which is qualitatively more aversive. Also, these same Prh-lesioned rats were capable of learning a flavor preference task a few weeks later (Ramos, 2020). Taken together, these data indicate that bilaterally IC and Prh-lesioned rats can differentiate between preferred and non-preferred solutions. Therefore, it is unlikely that this study's contralateral rats, with only unilateral lesions in each one of these structures, could have a discriminative/perceptive deficit that explains the disruption of taste neophobia.

The current study's results, a severe disruption of taste neophobia after Prh-IC disconnection, are comparable to those observed after lesions to each of these regions separately. As for the IC, some studies have shown that excitotoxic lesions to this region reduce taste neophobia in rats without affecting the attenuation of the neophobia, in parallel with the data obtained in this research. Thus, lesioned rats consume significantly more saccharin than control animals on the first exposure, with both groups reaching the same level at asymptote after repeated exposures (Lin, Roman, St. Andre, & Reilly, 2009; Moraga-Amaro, Cortés-Rojas, Simon, & Stehberg, 2014). Importantly, other studies have shown that in rats with IC lesions quinine palatability is higher than in controls in the first neophobic trial, indicated by larger lick cluster sizes (Lin, Arthurs, & Reilly, 2015). Consequently, IC-lesioned rats elevate the perceived hedonic value of the taste stimulus (quinine) and consume, during the first neophobia trial, over twice the amount consumed by the sham rats (see exp. 2, Lin et al., 2015). Thus, in agreement with the aforementioned authors, IC could be necessary to detect/process information related to the danger associated with a novel taste stimulus (Lin et al., 2015; Reilly, 2018b). Some studies with IC-lesioned rats subjected to CTA support this idea. Specifically, IC-lesioned animals acquired a CTA to a novel CS at a rate similar to that observed in control rats conditioned to a familiar CS taste with high palatability (Roman, Lin, & Reilly, 2009). According to Reilly and associates, this latent inhibition-like effect is compatible with the view that the IC is essential in

the detection of information related to the potential danger of unfamiliar foods. (Lin et al., 2015; Roman, Lin, & Reilly, 2009).

With respect to the Prh, although very few studies have been conducted, previous data from our lab, using a one-bottle (Ramos, 2015) or a two-bottle procedure (saccharin vs. water, Ramos, 2020), concur with the results of the current investigation. Specifically, as mentioned above, Prh lesions disrupted the initial neophobic response to 0.3% and 0.5% saccharin but a normal and robust taste neophobia was observed with 0.7% saccharin. A possible explanation for these results is that, in addition to a sweet component, saccharin has a bitter component (Horne, Lawless, Speirs, & Sposato, 2002; Kuhn et al., 2004). In consequence, a more concentrated saccharin solution (0.7%, for example) can be experienced by the rats as qualitatively aversive, and it is normally considered a non-preferred solution (Smith & Sclafani, 2002). In this regard, a recent study found that Prh excitotoxic lesions did not disrupt the initial neophobic response to a 3% cider vinegar solution, but that the lesioned rats did show a delay in the attenuation of neophobia (Morillas, Gómez-Chacón, & Gallo, 2017). Since vinegar has a sour taste and contains various acidic elements, it causes affectively negative reactions and, like the higher concentrations of saccharin, is avoided by animals (Schier & Spector, 2019). So, it may be that the type of gustatory stimulus used is decisive in whether or not the Prh is involved in the neophobic response. Both the study by Morillas et al. (2017) and our results with 0.7% saccharin (Ramos, 2015, exp. 1c; Ramos, 2020, exp. 1c) suggest that Prh has little influence on the neophobic response to aversive gustatory stimuli. However, in the case of sapid stimuli (0.3% saccharin, for example) Prh does seem critical, and the present study is the third time that we replicate the same results with different methodologies. Regarding the attenuation of neophobia, previous studies have implicated the Prh by demonstrating a delay in the attenuation of neophobia following intraPrh infusion of scopolamine (Gutiérrez, De la Cruz, Rodríguez-Ortiz, & Bermúdez-Rattoni, 2004), anisomycin (De la Cruz, Rodríguez-Ortiz, Balderas, & Bermúdez-Rattoni, 2008) or lidocaine (Ramos, 2020), just after saccharin consumption in trial 1. This suggests that, probably, within the Prh two neural mechanisms coexist, one related to the

initial neophobic response and one related to the plastic changes underlying neophobia attenuation. In other structures of the neural circuit that controls gustatory neophobia, specifically the basolateral amygdala and gustatory cortex, a recent study succeeded in affecting both processes simultaneously (Lin, Arthurs, & Reilly, 2018). The authors found that inactivation of each structure (by infusion of baclofen/muscimol) before presentation to a novel saccharin solution (0.5%) increased the amount of saccharin consumed in trial 1 (i.e., disrupted the neophobic response itself) and decreased intake in trial 2 (i.e., impaired memory consolidation, attenuation of neophobia). Despite this, our Prh excitotoxic lesions only affected, in the present and previous studies, the initial neophobic response. Unlike our results, the study by Morillas et al. (2017) did manage to selectively affect the attenuation of neophobia. Taken together, these studies suggest that Prh supports both processes, taste neophobia and attenuation of neophobia. However, the reasons for which our study and that of Morillas et al. (2017) affect different processes selectively are unknown, but they could be due to methodological differences. For one, the extent of the lesions is different in the two studies. For example, Morillas et al. reported that in some rats the lesions affected the piriform cortex. In relation to the foregoing, a recent study showed that the posterior piriform cortex of adult rats exhibited a higher number of c-Fos positive cells after exposure to the familiar flavor (3% cider vinegar solution) than after exposure to the novel flavor (Grau-Perales, Gómez-Chacón,, Morillas, & Gallo, 2019). Thus, a disruption of normal piriform activity could potentially delay attenuation of neophobia, as Morillas et al. (2017) found. Secondly, the flavor solution used in the two studies is qualitatively very different. In consequence, as suggested by the authors themselves (Morillas et al., 2017, p. 233), Prh lesions could have a greater impact on the attenuation of neophobia when a flavor with a strong odor component, such as vinegar, is used.

As regards the function of Prh in taste neophobia, although little research has been conducted in this area, some data raise the possibility that Prh may be involved in novelty detection. Some studies have shown that pharmacological inactivation of the Prh before the presentation of a novel taste (0.1% saccharin) disrupted the normal acquisition of CTA and lesioned animals

drank a larger amount of the CS than controls in the probe test 24 h after conditioning (Tassoni et al., 2000; Gutiérrez et al., 2004). In contrast, Prh inactivation immediately after intake of the CS did not produce a disruption, and a robust CTA, similar to the one observed in control rats, was observed (Tassoni et al., 2000). It would seem that inactivation of the Prh during the conditioning phase, which should be producing a disruption of taste neophobia, prevents the animals from correctly processing the CS as novel, reducing the strength of conditioning due to a latent inhibition-like effect. This view is supported by numerous studies demonstrating that many neurons in Prh respond more intensely to novel than to familiar stimuli, suggesting that this structure plays a relevant role in the processing of novelty (Xiang & Brown, 1998; Li, Miller, & Desimone, 1993; Albasser, Poirier, & Aggleton, 2010). Nonetheless, given that previous studies have implicated the Prh in some aspects of fear memory (Bucci, Saddoris, & Burwell, 2002; Kholodar-Smith, Boguszewski, & Brown, 2008), it cannot be ruled out that there is some Prh involvement in the processing of danger associated with a novel taste stimulus, meaning that this region might share some functions with IC.

In conclusion, the interaction between Prh and IC is critical for normal expression of taste neophobia. The present study shows that Prh-IC connections constitute a functional circuit. The precise operations carried out by this circuit are not fully understood. However, given the functions attributed to each of its components, Prh and IC, it is likely that such operations have to do with the processing of the novelty and the danger of stimuli. Finally, a previous study also demonstrated a functional relationship between the basolateral amygdala and IC (Lin & Reilly, 2012). This suggests that IC is a key region within the brain system underlying taste neophobia and that it interacts with different anatomical components of the circuit to organize an integrated neophobic response.

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FIGURE 1

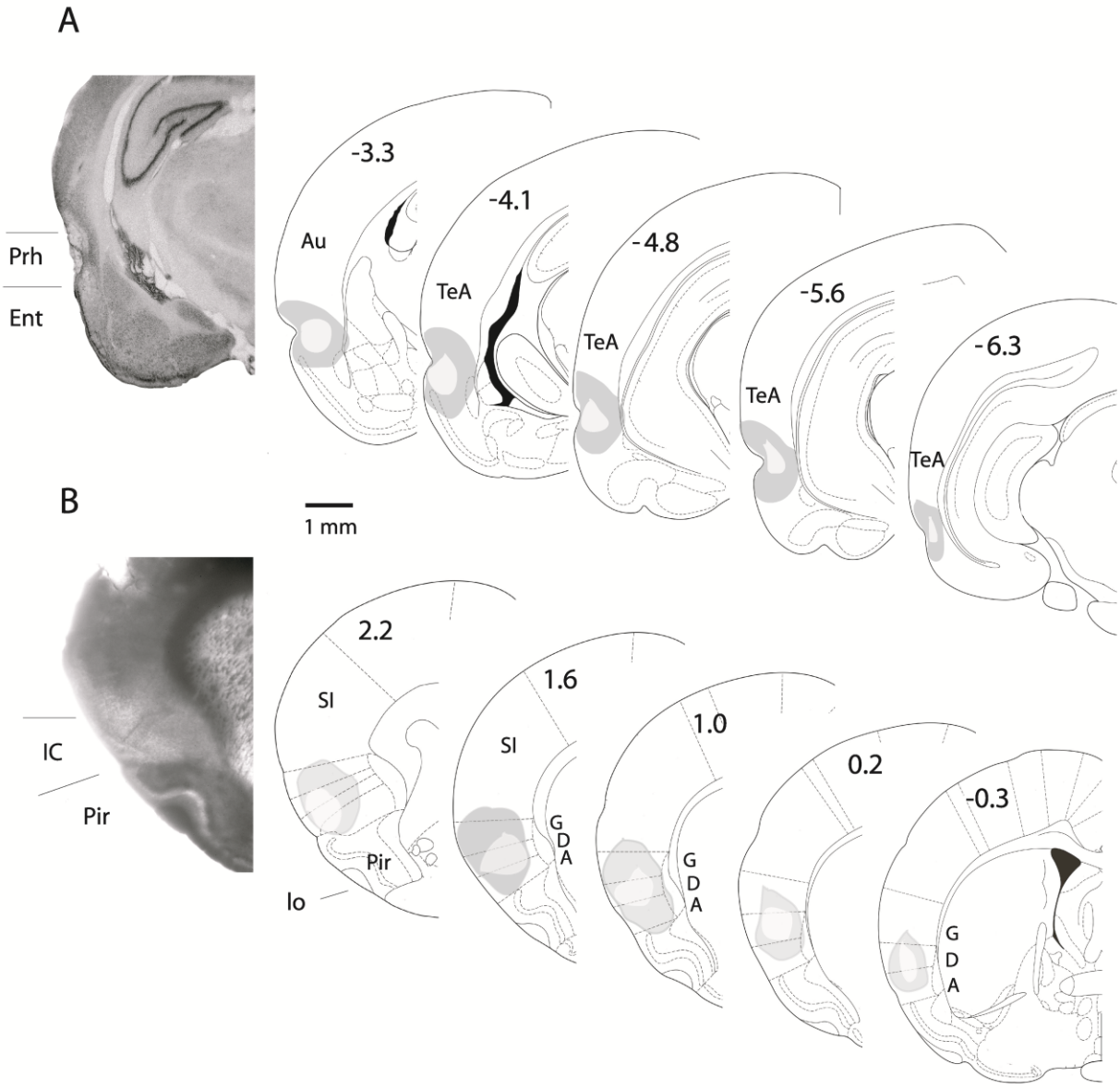

Figure 1. Photomicrographs showing representative lesions and serial reconstruction of the smallest (central white area) and largest (gray) excitotoxic lesions of the perirhinal (A) and insular (B) cortices. AP coordinates are shown in relation to bregma. Abbreviations: A, agranular insular cortex; Au, primary auditory cortex; D, dysgranular insular cortex; Ent, entorhinal cortex; G, granular insular cortex; IC, insular cortex; lo, lateral olfactory tract; Pir, piriform cortex; Prh, perirhinal cortex; SI, primary somatosensory cortex; TeA, temporal association cortex.

FIGURE 2

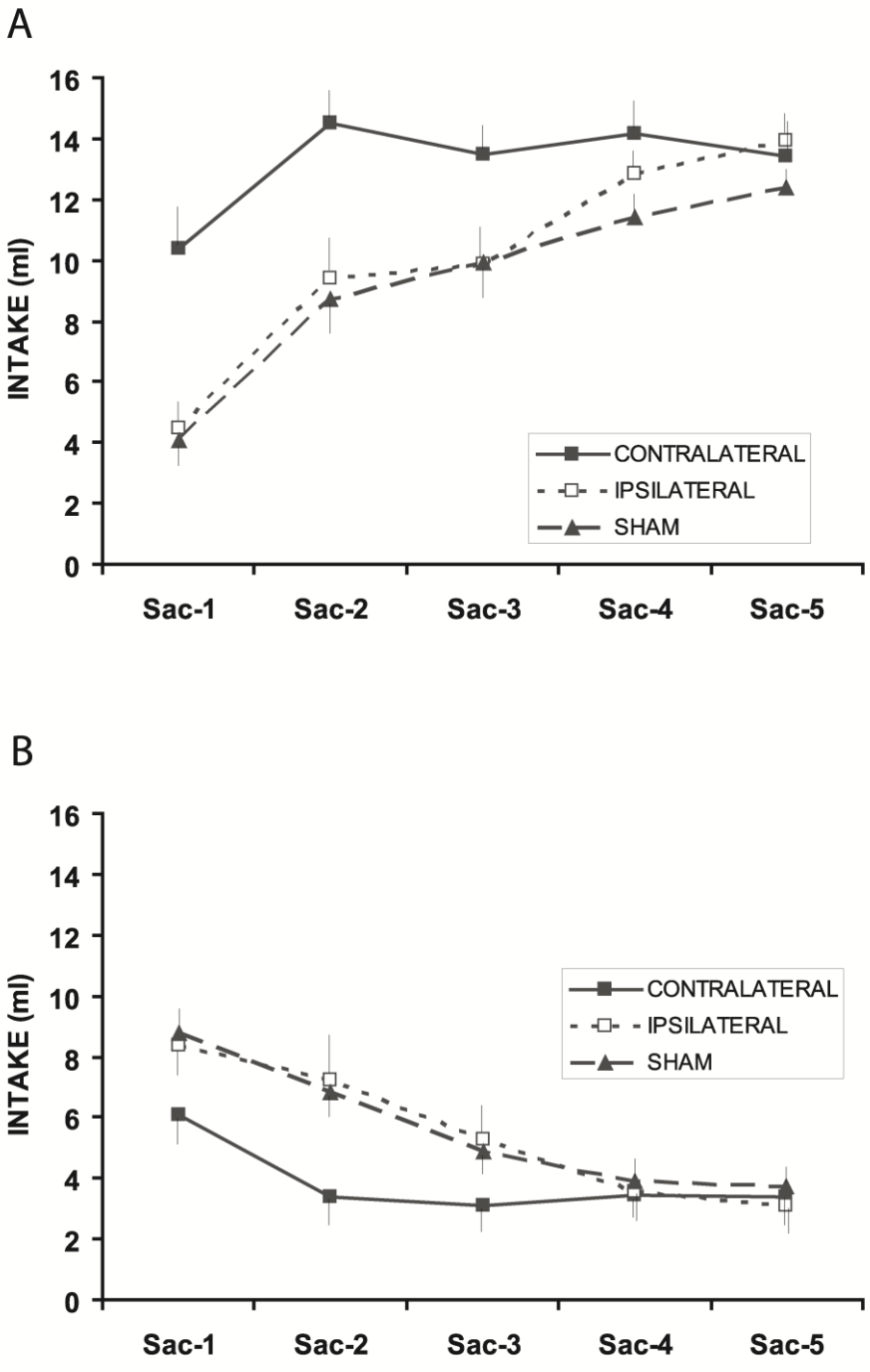

Figure 2. A) Mean (±SEM) saccharin intake across the 5 successive taste trials. B) Mean (±SEM) water intake during the afternoon across the 5 successive experimental days.