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Effects of Insular Cortex Lesions on Conditioned Taste Aversion and Latent Inhibition in the Rat

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Abstract

The present study tested the hypothesis that lesions of the insular cortex of the rat retard the acquisition of conditioned taste aversions (CTAs) because of an impairment in the detection of the novelty of taste stimuli. Demonstrating the expected latent inhibition effect, nonlesioned control subjects acquired CTAs more rapidly when the conditioned stimulus (0.15% sodium saccharin) was novel rather than familiar (achieved by pre-exposure to the to-be-conditioned taste cue). However, rats with insular cortex lesions acquired taste aversions at the same slow rate regardless of whether the saccharin was novel or familiar. The pattern of behavioural deficits obtained cannot be interpreted as disruptions of taste detection or stimulus intensity, but is consistent with the view that insular cortex lesions disrupt taste neophobia, a dysfunction that consequently retards CTA acquisition because of a latent inhibition-like effect.

Keywords: conditioned taste aversion; gustatory neophobia; insular cortex; latent inhibition; rat

Introduction

Numerous studies report that damage/inactivation of the insular cortex (IC) disrupts acquisition of conditioned taste aversion (CTA) (e.g. Braun *et al*., 1972; Bermúdez-Rattoni & McGaugh, 1991; Gallo *et al*., 1992; Cubero *et al*., 1999; Fresquet *et al*., 2004), whereas others have found that IC lesions spare CTA (e.g. Mackey *et al*., 1986; Roldan & Bures, 1994; Yamamoto *et al*., 1995; Sakai & Yamamoto, 1999). Among the studies that report impairments, there is a lack of consensus on the nature of the deficit.

Many of the studies cited above used a one-trial conditioning procedure, a design choice that may not provide sufficient opportunity to allow proper determination of the nature of a lesion-induced CTA deficit (Reilly & Bornovalova, 2005). This shortcoming is exacerbated when combined with inadvertent pre-exposure to the taste cue [conditioned stimulus (CS)] prior to conditioning, because CTAs are more slowly acquired by a familiar and safe (i.e. pre-exposed) CS than by a novel CS [a phenomenon termed latent inhibition; e.g. Lubow (1989, 2008)]. In the absence of appropriate control (i.e. nonpre-exposed) animals, it is not possible to determine whether any obtained lesion-induced deficit should be interpreted as a disruption of the mechanisms involved in CTA, latent inhibition, or both.

Recently, we reported that IC lesions attenuated CTA but had no influence on the acquisition of a conditioned odour aversion (Roman *et al*., 2006). Because the CTA deficit was most pronounced on the first conditioning trial (lesioned rats drank twice as much saccharin as control subjects), the disruption seemed to be best interpreted as a decreased ability to process some aspect of the taste stimulus, perhaps a failure to recognize taste novelty.

When conducting a CTA latent inhibition study with a preparation that might show disrupted responsivity to novel taste stimuli, it is important to minimize the potentially confounding influence of a lesion-induced elevation of consumption on learning by limiting the amount of CS that can be ingested during both the pre-exposure and conditioning trials (e.g. Reilly *et al*., 2003; St Andre & Reilly, 2007). Furthermore, with the exception of Kiefer & Braun (1977), all of the studies that have examined CTA acquisition in pre-exposed insular cortex-lesioned (ICX) rats failed to include a group of nonpre-exposed control rats (e.g. Hankins *et al*., 1974; Kiefer *et al*., 1984; Dunn & Everitt, 1988; Bermúdez-Rattoni & McGaugh, 1991). Thus, unambiguous interpretation of the effects of IC lesions on CTA acquisition in these reports is not possible. Although the Kiefer and Braun experiment did include nonpre-exposed control subjects, their design did not control for lesion-induced intake differences during the pre-exposure and conditioning trials, and nonselective lesions were employed. To more completely evaluate the role of the IC in CTA acquisition, the present study manipulated CS novelty in a design that involved capped intake and multiple conditioning trials in rats with neurotoxic lesions.

Materials and Methods

Experimental subjects

The subjects used in this experiment were 42 male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA). Rats were housed individually in hanging steel mesh cages

with *ad libitum* food and water except where otherwise noted. The vivarium was kept on a 12-h light/dark schedule, with lights on at 7:00 a.m. Experimental treatments and procedures were performed during the light phase of this cycle. All rats were between 290 g and 320 g at the time of surgery. The subjects were treated in accord with the ethical guidelines established by the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, and the experimental procedures were approved by the Institutional Animal Care and Users' Committee of the University of Illinois at Chicago.

Surgery

Twenty-two rats received bilateral lesions of the IC (group ICX). They were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and fixed into a stereotaxic instrument (ASI, Warren, MI) with atraumatic earbars. Body temperature was monitored with a rectal thermometer and maintained at 37 °C with a heating pad (Harvard Apparatus, Holliston, MA). Bupivacaine (0.25%; Hospira, Lake Forest, IL) was subcutaneously injected into the scalp prior to a midline incision that exposed the cranial sutures. The skull was levelled between bregma and lambda, and trephine holes were drilled over the IC. A glass micropipette (tip ∼75 μm) containing 0.15 m*N*-methyl-d-aspartate (St Louis, MO) was lowered into the IC in each hemisphere, and two iontophoretic infusions were delivered per hemisphere (site 1, 10-min infusion at AP +1.2, ML ± 5.2, DV −5.0; site 2, 6-min infusion at AP +1.2, ML ± 5.2, DV −4.3) using a Midgard precision current source (Stoelting, Wood Dale, IL). After the fourth infusion, the incision was closed with wound clips, and the rats were returned to their home cage once they had recovered from the anaesthesia. Control rats (group SHAM; n = 20) were anaesthetized using sodium pentobarbital, but did not undergo any further surgical treatments. All rats were given a minimum of 7 days to recover from surgery before the experiment began.

Apparatus

All experimental manipulations were performed in the home cages. Fluids were presented in inverted 100-mL Nalgene graduated cylinders with silicone stoppers and steel drinking tubes. Fluid consumption was recorded to the nearest 0.5 mL.

Procedure

After recuperation, the rats were placed on a water restriction schedule that permitted 15 min of access daily. This level of deprivation was used to maintain comparability with our previous study of the influence of IC lesions on CTA and conditioned odour aversion (Roman *et al*., 2006) and our work on latent inhibition in CTA (Reilly *et al*., 2003; St Andre & Reilly, 2007). Once water intake stabilized, the rats were divided into groups based on lesion (SHAM or ICX) and whether they would be pre-exposed (Familiar condition) or not (Novel condition) to the CS before conditioning: SHAM-Familiar (n = 10), SHAM-Novel (n = 10), ICX-Familiar (n = 11), and ICX-Novel (n = 11). Rats in the Familiar condition were given access to 15 mL of the future CS [0.15% sodium saccharin (w/v)] on days 1–5, whereas rats in the Novel condition received an equivalent amount of water each day. On day 6, all rats received 10 mL of the CS, followed, 30 min after initial placement of the stimulus bottles, by an intraperitoneal injection of the unconditioned

stimulus (US; 0.15 m lithium chloride injected at 1.33 mL/100 g body weight). This CS–US pairing was repeated on days 9, 12, 15, and 18, with 2 days of 15-min access to water on the intervening days. On day 21, all rats received a test trial consisting of 15 min of access to the CS (the US was omitted because it was superfluous).

Histology

After the behavioural testing was completed, the rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg) and perfused transcardially with physiological saline and 4% buffered formalin. The brains were extracted and stored in 4% buffered formalin for 2 days, then transferred into 20% sucrose for at least 2 days. The brains were frozen, sliced on a cryostat at 50 μm, mounted on gelatin-coated slides, and stained with cresyl violet. The brain sections were evaluated with a light microscope (Zeiss Axioskop 40). Drawings of the lesions were made on diagrams obtained from the Paxinos & Watson (2005) atlas, and a representative photomicrograph of an IC lesion was taken using Q-Capture software (Quantitative Imaging Corporation, Burnaby, BC).

Data analysis

Using the Statistica software package (StatSoft, Tulsa, OK), the significance of the behavioural data (volume of fluid consumed) was assessed with analysis of variance (anova; the alpha level was set at $P < 0.05$). Fluid intake data are presented as the mean ± SEM.

Results

Anatomical analysis

Located along the dorsal bank of the rhinal fissure on either side of the middle cerebral artery, the gustatory region of the IC is ∼0.5 mm wide dorsoventrally and ∼2.5 mm long anteroposteriorly (Kosar *et al*., 1986; Nakashima *et al*., 2000). The extent of the neurotoxic lesions was determined by the presence of gliosis and the absence (or shrivelling) of cell bodies. Rats with lesions that were unilateral (two Novel, one Familiar) or subtotal (two Familiar) were excluded from the study. The remaining rats (nine Novel, eight Familiar) had lesions that were bilaterally well placed within the IC with minimal encroachment into the claustrum, external capsule, and piriform cortex. Serial schematic reconstructions of the largest (grey) and smallest (black) lesions of rats that were included in the statistical analyses are shown in Fig. 1A. Figure 1B shows a photomicrograph of a representative IC lesion. Fig. 1C shows the same region in an intact brain. The IC lesions in the present study were comparable in location, although slightly larger in extent, to those in our earlier report (Roman *et al*., 2006).

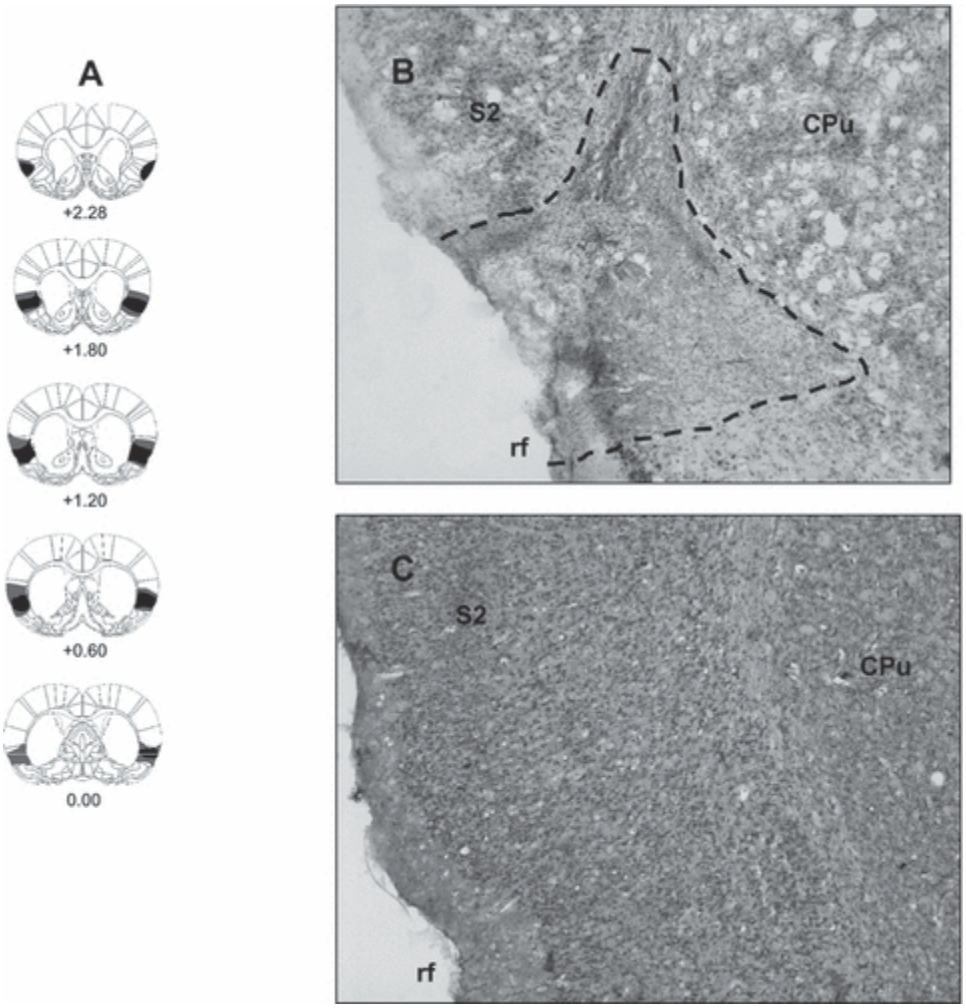

Figure 1. (A) Serial reconstructions of the smallest (black) and largest (grey) neurotoxic lesions of the insular cortex on diagrams adapted with permission from the Paxinos & Watson (2005) atlas. The numbers (0.00 mm, +0.60 mm, +1.20 mm, +1.80 mm, +2.28 mm) beneath each diagram refer to the anteroposterior coordinates relative to bregma. (B) Representative neurotoxic lesion of the insular cortex in the left hemisphere of an experimental rat taken at ~0.8 mm anterior to bregma. (C) Corresponding section through the insular cortex of a neurologically intact subject. CPu, caudate putamen; rf: rhinal fissure; S2, secondary somatosensory cortex.

Behavioural analysis

On the day prior to the first saccharin pre-exposure trial, the mean (±SEM) water intake (mL) for each group was as follows: SHAM-Novel, 20.4 ± 0.89; SHAM-Familiar, 19.7 ± 0.96; ICX-Novel, 18.1 ± 0.74; ICX-Familiar, 19.0 ± 0.92. A lesion (SHAM vs. ICX) × condition (Novel vs. Familiar) anova confirmed that there were no significant ($F < 1$) intergroup differences in water consumption prior to the saccharin pre-exposure trials.

As is evident from inspection of the pre-exposure data shown in Fig. 2, the SHAM-Familiar subjects drank less saccharin on trial 1 than on trials 2–5, whereas the ICX-Familiar rats drank the maximal amount (15 mL) on each pre-exposure trial. These impressions were confirmed with an

anova that found significant main effects of lesion ($F_{1,16}$ = 6.68, P < 0.05) and of pre-exposure trials ($F_{4,64}$ = 7.496, P < 0.05), as well as a significant lesion × trials interaction ($F_{4,64}$ = 7.496, P < 0.05). These data indicate the occurrence of a neophobic response in the SHAM subjects, and the absence of neophobia in the ICX rats, on the first saccharin pre-exposure trial. The SHAM and ICX subjects in the Novel condition drank 15 mL of water on each of the five pre-exposure trials.

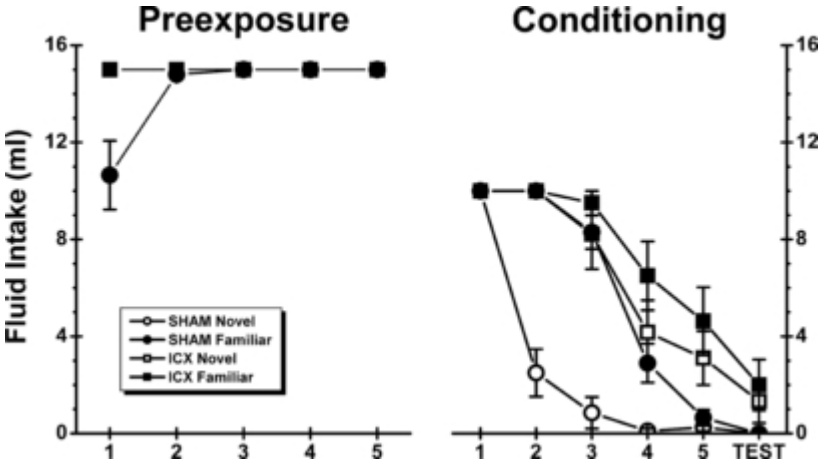

Figure 2. Mean (±SEM) saccharin intake for neurologically intact (SHAM) subjects and insular cortex-lesioned (ICX) rats during the pre-exposure and conditioning phases of the experiment.

The conditioning data summarized in Fig. 2 suggest that SHAM subjects displayed a pronounced latent inhibition effect, whereas rats in the ICX-Novel and ICX-Familiar groups acquired the saccharin aversion at the same slow rate. An anova conducted on the conditioning and test data confirmed significant main effects of condition ($F_{1,165}$ = 19.60, P < 0.05), lesion ($F_{1,165}$ = 38.93, P < 0.05), and trials ($F_{5,165}$ = 146.13, P < 0.05), as well as a significant condition × lesion × trials interaction ($F_{5,165}$ = 9.82, P < 0.05). To follow up the significant triple interaction, separate condition × trial anovas revealed that SHAM-Familiar subjects acquired the saccharin CTA significantly more slowly than SHAM-Novel rats ($F_{5,90}$ = 30.93, P < 0.05). On the other hand, as indicated by the absence of a significant condition × trials interaction (F < 1), ICX-Novel rats acquired the saccharin CTA as slowly as the ICX-Familiar rats. Finally, ICX and SHAM rats were compared on each trial in the Novel condition, and separately in the Familiar condition. When the CS was novel, ICX rats consumed more saccharin than SHAM subjects on trials 2, 3, 4, and 5 (P < 0.05), but not on trial 1 (F < 1; when intake was maximal in both groups) or the test trial (P > 0.10). Thus, IC lesions retarded but did not prevent CTA acquisition. When the CS was familiar, SHAM and ICX rats drank equivalent amounts of saccharin on the first three trials (P > 0.05), but on trials 4 and 5 and the test trial ICX rats consumed more than SHAM subjects (P < 0.05). Once again, then, IC lesions attenuated but did not prevent CTA acquisition.

Discussion

The neurologically intact subjects displayed a noticeable neophobic reaction on their initial exposure to saccharin during the pre-exposure phase of the experiment. Furthermore, saccharin pre-exposure attenuated CTA acquisition in these rats. That is, SHAM-Familiar subjects showed

the expected latent inhibition effect relative to the SHAM-Novel subjects. On the other hand, the ICX-Familiar rats drank maximal amounts of saccharin during each pre-exposure trial (and significantly more saccharin than SHAM-Familiar subjects on pre-exposure trial 1) and the ICX-Novel rats developed a CTA at the same slow rate as the ICX-Familiar rats. The results also show that both groups of ICX rats acquired CTAs at a significantly slower rate than the SHAM-Familiar subjects. These findings were obtained using saccharin as the CS. It will, of course, be important to test other, qualitatively different, taste cues in the same experimental design in order to establish the generality of the present results.

The present results are consistent with studies that have found a CTA acquisition deficit in ICX rats (e.g. Nerad *et al*., 1996; Roman *et al*., 2006) but conflict with those that have reported no deficits (e.g. Yamamoto *et al*., 1995; Sakai & Yamamoto, 1999). We believe that these different CTA results are not dependent upon the length of the interstimulus intervals (the former studies used CS–US delays of 10–20 min, whereas the latter studies used a nominal no-delay design), but are best explained in terms of the location and extent of the IC lesions. Kosar *et al*. (1986) identified the boundaries of the gustatory region of the IC as extending for ∼1.0 mm anterior and posterior to the intersection of the rhinal fissure and the middle cerebral artery (∼0.8 mm anterior to bregma). Thus, the gustatory region of the IC extends from approximately +1.8 mm to −0.2 mm AP. In the Sakai & Yamamoto (1999) study, the maximal area of common damage in all ICX rats was between +2.7 mm and +1.7 mm anterior to bregma, and in the Yamamoto *et al*. (1995) study, the representative IC lesions were centred at +1.8 mm AP. In both studies, then, the critical gustatory region would seem to have been less completely damaged than in the studies by Nerad *et al*. (1996) and Roman *et al*. (2006), and in the present report.

The only lesion-induced deficit that was not expected in the present study concerns the difference between the two groups of ICX rats and the SHAM-Familiar subjects; our experimental hypothesis (that ICX rats treat novel taste stimuli as if they are familiar) anticipated that these three groups should acquire CTAs at the same rate. How is this disparity to be explained? Two dysfunctions, which concern lesion-induced disruptions of taste detection or stimulus intensity, can immediately be ruled out on the basis of the present results. First, if IC lesions rendered a rat completely ageusic, then taste aversion learning would be impossible. It is clear, then, that ICX rats were not blind to taste stimuli, as CTA acquisition was attenuated, but not prevented. The second dysfunction would mean that IC lesions reduce the perceived intensity of taste stimuli. Although this could readily explain a lesion-induced overconsumption of saccharin on the first pre-exposure trial (lower concentrations of saccharin evoke less neophobia than do higher concentrations), it would not mean that ICX-Novel rats would acquire the taste aversion as slowly as the ICX-Familiar subjects [latent inhibition occurs even with low concentrations of saccharin; for example, De La Casa & Lubow (2002) used 0.04% saccharin]. Finally, it might be noted that there is empirical evidence that IC lesions do not disrupt basic taste perception (Braun *et al*. 1982).

One might, of course, suggest that the behavioural deficit during pre-exposure is the product of the disruption of a qualitatively different IC mechanism than the behavioural deficits found during conditioning. Furthermore, one might argue that each group of ICX animals displayed abnormal performance during the conditioning phase: impaired CTA acquisition in the ICX-Novel rats and

enhanced latent inhibition in the ICX-Familiar subjects. Does the pattern of behavioural deficits found during conditioning reflect the disruptions of two different IC mechanisms or can a single dysfunctional IC mechanism account for both deficits? That is, is the apparent enhanced latent inhibition effect in the ICX-Familiar rats better understood as impaired CTA acquisition (as shown in the ICX-Novel rats) rather than merely further retardation by stimulus pre-exposure? Thus, depending on one's interpretation of the conditioning data, it is possible to argue that IC lesions disrupted two or perhaps three mechanisms in the present study.

It is not clear, however, that parsimony needs to be abandoned in order to explain the full pattern of deficits in ICX rats. Although the present latent inhibition design precludes a determination of the magnitude of the lesion-induced deficit on first exposure to saccharin, a recent CTA experiment from our laboratory, which employed the same deprivation schedule and saccharin concentration as the present study (Roman *et al*., 2006), found that ICX rats consumed twice as much saccharin on the first conditioning trial than the SHAM subjects (~21.0 mL vs. ~9.0 mL, respectively). Assuming this analysis to be valid, an alternative account of the present pattern of results may be entertained, an account that focuses on the performance of the SHAM-Familiar subjects, not that of the ICX rats. That is, perhaps the five pre-exposure trials were insufficient to fully familiarize SHAM subjects with saccharin to the level at which it was perceived by the ICX rats. In turn, the SHAM-Familiar subjects would be expected to acquire CTAs more rapidly than each group of ICX rats.

This analysis has two virtues: it is readily testable and, if correct, the pattern of results in the ICX rats of the present study is entirely consistent with our experimental hypothesis that IC lesions disrupt CTA acquisition as a secondary consequence of an impairment in the detection of the novelty of taste stimuli. Taste neophobia (Barnett, 1963) refers to the fact that rats are reluctant to eat a novel food because of the unknown, and potentially toxic, postingestive consequences of consumption. However, in the absence of aversive gastrointestinal feedback, neophobia eventually dissipates following repeated exposures to the now familiar and safe food (Barnett, 1963; Domjan, 1977; Corey, 1978). If our analysis of the present results is valid, then questions concerning the role of the IC in CTA need to be recast in terms of the role of the IC in taste neophobia. To our knowledge, however, there has been no published study that has systematically investigated the effects of IC lesions on taste neophobia. Such a study is currently underway in our laboratory, and seeks to evaluate not only the magnitude of the taste neophobia deficit but also the specificity of the deficit to the modality of taste.

The results of the present experiment not only help to clarify the role of the IC in CTA, but also enhance our understanding of the neurological system underlying taste aversion learning. It has been shown that rats with parabrachial nucleus lesions are unable to learn CTAs [for reviews, see Reilly (1999, 2008)]. The parabrachial nucleus is a brainstem nucleus that projects to several forebrain structures, including the thalamus, lateral hypothalamus, amygdala, bed nucleus of the stria terminalis and IC. Other research has demonstrated that transecting all axons between the forebrain and brainstem also prevents the development of CTAs (Grill & Norgren, 1978). Together, these lines of evidence suggest that a parabrachial nucleus interaction with one or more forebrain structures is essential for the occurrence of normal CTA. Many lesion studies have been

conducted to test this analysis, but there appears to be little convincing evidence that any of the aforementioned nuclei are critical for CTA acquisition in manner comparable to the parabrachial nucleus (Roman *et al*., 2006). Indeed, to our knowledge, only the basolateral region of the amygdala (BLA) and IC show any significant involvement in CTA. In both cases, it should be noted, the deficit is retardation, not abolition of taste aversion learning.

The present results bear a strong resemblance to the data obtained from rats with lesions of the BLA tested with the same latent inhibition procedure (St Andre & Reilly, 2007; Reilly & Bornovalova, 2005). In the experiment with BLA-lesioned rats, the SHAM-Familiar and BLA-lesioned-Familiar groups acquired aversions at the same rate (that is, latent inhibition was demonstrated). As is typical, SHAM-Novel subjects rapidly developed aversions, but the BLA-lesioned-Novel rats acquired aversions at an intermediate rate relative to the other three groups (given that the lesions in these rats were subtotal, it is possible that the behavioural impairment would have been more complete if the lesions were larger). These results support the view that the BLA and IC perform similar roles in CTA. Specifically, both structures are important for the accurate detection/recognition of taste novelty.

That lesions of the IC and BLA have similar influences on CTA is hardly surprising, as the two areas are anatomically interconnected (Krettek & Price, 1977; Ottersen, 1982; Shi & Cassell, 1998). However, similarities in function and anatomical connectivity do not guarantee redundancy. If these two areas did perform redundant functions, then lesions of either structure might not lead to meaningful behavioural deficits, because the other structure would be available to execute that behaviour. Rather, the similarities of deficits consequent to lesions of the BLA and IC suggest that the two structures are functionally interdependent. This analysis offers direction to future studies that might profitably examine the effects of combined BLA and IC lesions or asymmetrical lesions of the two structures on taste neophobia and CTA. In this context, it might be noted that Yamamoto (1993) found that dual lesions of the IC and amygdala interrupt CTA acquisition to a greater degree than lesions of either structure alone. Also, Bielavska & Roldan (1996) found that contralateral tetrodotoxin inactivations of the IC and amygdala interrupted CTA more than ipsilateral inactivations. Repeating these studies with lesions limited to the BLA rather than multiple amygdala subnuclei, and utilizing multiple conditioning trials to more carefully assay the nature of the deficit, would benefit our understanding of the interplay between these two structures.

The results of the present latent inhibition study support the experimental hypothesis that the IC is essential for the detection/recognition of taste novelty. ICX rats treat a novel taste stimulus as if it is familiar, and consequently CTA is retarded because of a latent inhibition-like effect. By this analysis, the IC has an important but nonessential role in the neural system underlying taste aversion learning.

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Abbreviations

BLA

basolateral amygdala

CS

conditioned stimulus

CTA

conditioned taste aversion

IC

insular cortex

ICX

insular cortex-lesioned

US

unconditioned stimulus.

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