Published in final edited form as:

Eur J Neurosci. 2007 September ; 26(6): 1692–1700.

Insular Cortex Lesions Fail to Block Flavor and Taste Preference Learning in Rats

Khalid Touzani and Anthony Sclafani

Department of Psychology, Brooklyn College and The Graduate School, City University of New York, Brooklyn, NY 11210 USA

Abstract

The role of the insular cortex (IC) in learning to associate orosensory cues with the oral and post-oral properties of carbohydrate was examined. Rats with either small (gustatory region) or large (gustatory and visceral regions) ibotenic acid lesions of the IC learned to prefer flavors (Experiments 1 & 3) and taste mixtures (Experiments 2 & 4) paired with intragastric (IG) infusions of maltodextrin. The rats with large IC lesions also learned a preference for a flavor cue paired with the sweet taste of fructose (Experiment 5). In fact, they showed enhanced conditioning and retarded extinction compared to controls. Collectively, these data provided no evidence that IC is essential for flavor preference learning based on associations between the orosensory cues and the oral and post-oral reinforcing properties of nutrients.

Keywords

carbohydrate; conditioning; ibotenic acid; extinction

Introduction

Beside their innate predispositions to accept some foods (e.g., sweet tasting) and reject others (e.g., bitter tasting), animals readily acquire new food preferences and aversions through associative learning processes. If animals consume an unfamiliar food and experience visceral malaise, they readily learn to avoid the food's flavor (i.e., its taste, odor, texture) at subsequent exposures. This form of learning, which is typically referred to as conditioned taste aversion (CTA), is well documented (Bures et al., 1998). Animals also learn to prefer the flavor of foods and fluids that are associated with positive postingestive nutritional consequences. This is documented by numerous studies showing that rats acquire preferences for flavored solutions that either contain a nutrient or are paired with intragastric (IG) infusions of nutrients (Capaldi, 1996; Sclafani, 1999).

Flavor aversion and preference learning are forms of classical conditioning in which the flavor cue (conditioned stimulus, CS) is associated with the postingestive consequences of a toxic or nutritious substance (unconditioned stimulus, US). Such learning requires the neural integration of orosensory and viscerosensory information and the formation of long-term flavor memories. The brain mechanisms that mediate flavor aversion learning, or more specifically conditioned taste aversions, have been extensively investigated (Bures et al., 1998; Scalera, 2002). Impaired flavor aversion learning has been reported with lesions of a variety of structures, including the parabrachial nucleus (PBN), amygdala (AMY) and insular cortex (IC), although the deficits obtained with AMY and IC lesions are not as profound as those obtained

with PBN lesions (Nachman & Ashe, 1974; Bermúdez-Rattoni et al., 1983; Dunn & Everitt, 1988; Yamamoto et al., 1994; Sakai & Yamamoto, 1999).

Compared to flavor aversion learning, less is known about the neural substrates of flavor preference learning. Lesion studies demonstrated that PBN integrity is critical for nutrient-conditioned preferences when taste is the primary CS (Sclafani et al., 2001), whereas the AMY is essential for preference learning when the primary cue is a complex flavor with both gustatory and olfactory components (Touzani & Sclafani, 2005). The IC is another possible site for the mediation of flavor preference learning given that it receives gustatory, olfactory and visceral sensory inputs (Saper, 1982; Hanamori et al., 1998; Fu et al., 2004). Furthermore, it is hypothesized that the integration of gustatory and olfactory information necessary for the perception of flavor occurs in agranular insular cortex (Shipley & Geinisman, 1984; Cinelli et al., 1987).

A key finding implicating the IC in nutrient-conditioned flavor preferences is the observation that rats developed a preference for a flavored solution that was paired with electrical stimulation of the IC (Cubero & Puerto, 2000). Although electrical stimulation of other brain areas (i.e., lateral hypothalamus) are also reported to condition flavor preferences (Ettenberg & White, 1978), there are two notable aspects to the IC findings. First, the IC stimulation failed to support operant self-stimulation indicating that the conditioned flavor preference was not due to activation of a general “reward” circuit. Second, the IC stimulation conditioned a flavor preference even though there was a 15-min delay between removal of the flavored solution and the onset of IC stimulation. A special feature of flavor-nutrient preference learning is that, like conditioned flavor aversions, it tolerates long delays between the CS and US (Baker & Booth, 1989; Pérez et al., 1999; Touzani & Sclafani, 2002b). Thus, one interpretation of the preference learning conditioned by IC electrical stimulation is that the stimulation evoked a neural representation of positive viscerosensory effects produced by nutrients (Cubero & Puerto, 2000).

In view of the above findings, the present study determined if an intact IC is necessary for animals to acquire a nutrient-conditioned flavor preference. This was accomplished by training rats with IC lesions or sham lesions to associate flavor cues with IG infusions of a carbohydrate (maltodextrin). The lesions were either limited to the rostral (or gustatory) portion of the IC or included both the gustatory and visceral regions of the IC. Both flavor (olfactory + taste) and taste stimuli were used as CSs to determine if any lesion effects were related to the sensory nature of the CS. The nature of the US was also varied in that one experiment paired the CS flavor with an orally consumed sweet carbohydrate (fructose) rather than IG maltodextrin infusions.

Methods

Subjects

The subjects were 51 adult male Sprague-Dawley rats (28 obtained from Charles River Laboratories, Wilmington, MA and 23 bred in our laboratory from stock obtained from Charles River Laboratories); they weighed 350-470 g at the time of brain surgeries. The rats were individually housed in wire-mesh cages in a vivarium maintained at 21° C and under a 12:12 light:dark cycle (lights on at 0800h). They were fed powdered food (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and tap water. The research followed the guidelines and protocols approved by Brooklyn College Animal Care Committee required by NIH Animal Welfare Act.

Surgery

The rats were anesthetized with intraperitoneal injection of a ketamine hydrochloride (63 mg/Kg) and xylazine (9.4 mg/Kg) mixture and held in a Kopf stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line. Bilateral lesions of the insular cortex were made by microinjections of ibotenic acid (Sigma Chemical, St. Louis, MO) dissolved in vehicle (sodium phosphate buffer, pH 7.2) at a concentration of 10 µg/µl. The injections were made using a glass micropipette (tip diameter: 0.1 mm) that was connected to a Hamilton 1-µl microsyringe secured firmly and attached to a stereotaxic carrier. The controlled advancement of the syringe plunger was accomplished using a 25-mm micrometer drive. Rostral lesions of the insular cortex (rICx, n = 14) were made at the following coordinates: 1.2 mm anterior to Bregma, 5.3 mm lateral to the sagittal suture and 6.0 mm ventral from the surface of the skull. A volume of 0.5 µl was injected in each hemisphere. The injection lasted 5 min and 5 more min elapsed before the removal of the micropipette. Large lesions of the insular cortex (ICx, n = 18) were made by successively positioning the micropipette at the following coordinates: rostral injection: 2.0 mm anterior to Bregma, 5.6 mm to each side of the midline and 6.4 mm below the skull surface, 0.5 µl volume; medial injection: 0.6 mm anterior to Bregma, 6.2 mm to each side of the midline and 6.6 mm below the skull surface, 0.4 µl volume; caudal injection: -1.2 mm anterior to Bregma, 6.2 mm to each side of the midline and 6.9 mm below the skull surface, 0.4 µl volume. Using the same procedures, other rats were injected with the vehicle of the neurotoxin and served as sham-lesioned group (Sham, n = 19). Histological verification of the lesions was performed as described by Touzani and Sclafani (2001).

Three weeks following brain surgery, the rats were anesthetized and fitted with a gastric catheter connected to a Luer-lock assembly fixed to the skull as previously described (Touzani & Sclafani, 2001). Intramuscular penicillin (30,000 U) was given following both brain and gastric surgeries.

Apparatus

As detailed by Touzani and Sclafani (2001), training and testing occurred in plastic cages that gave the rats access to one or two stainless steel drinking spouts. The spouts were attached to drinking bottles mounted on motorized holders that positioned the spouts at the front of the cage at the start of the sessions and retracted them at the end of the sessions. Licking behavior was monitored by an electronic lickometer interfaced to a microcomputer that activated a syringe pump as the rat drank. Plastic tubing connected the pump to the rat's gastric catheter via the Luer-lock assembly. The infusion rate was 1.3 ml/min and the ratio of oral intake and infusion volume was maintained at approximately 1:1 by the computer software.

Test solutions

The conditioned stimuli were fruit-flavored (0.05% Kool-Aid, Kraft Foods, Rye Brook, NY) saccharin (0.2% sodium saccharin, Sigma, St. Louis, MO) solutions in Experiments 1, 3 and 5. In Experiments 2 and 4, taste mixtures were used containing 0.03% sucrose octaacetate + 0.2% saccharin ("bitter-sweet") and 2% NaCl + 0.2% saccharin ("salty-sweet"). In Experiments 1-4 one flavor or taste (the CS+) was paired with IG infusions of 16% maltodextrin (Maltrin QD M580, Grain Processing Corp., Muscatine, IA); the other flavor (the CS-) was paired with IG water infusions. The specific flavor-infusion pairs were counterbalanced across subjects. In Experiment 5, the rats were trained with a CS+/US solution that contained 8% fructose in addition to Kool-Aid flavor and saccharin and with a CS- solution that contained only flavor and saccharin. There were no IG infusions in this experiment. All solutions were prepared with tap water.

Procedures

Prior to brain surgery, the rats were familiarized with unflavored 0.2% saccharin solution by giving them ad libitum access to the saccharin solution along with water and powdered chow in their home cages for three days. Three weeks following brain surgery, the rats were again given ad libitum access to the saccharin solution along with water and powdered chow in their home cages for two days. Beginning two weeks after gastric surgery, the rats were adapted to the test cages and procedure. They were first housed in the test cages overnight with ad lib access to 0.2% saccharin solution, water and food; the saccharin and water bottles were automatically positioned to the front of the cages for 30 min every hour. Then the rats were placed on a food restriction schedule and maintained at 85% of their ad libitum body weights. They were adapted to drink the saccharin solution in the test cages during nine daily 30-min sessions. During the last four of these sessions, the rats were connected to the infusion system and were given IG water infusions as they drank the saccharin solution.

Statistical analysis

CS intakes were measured to the nearest 0.1 g and the data were analyzed using standard analyses of variance (ANOVA) procedures. Oral intakes during training and preference testing were averaged over 2-, 3- or 4-day blocks. Individual comparisons were evaluated using simple main effects tests or *t*-test when appropriate. Two-bottle preference data were also expressed as percent CS+ intake $[(\text{CS+ intake} / \text{total intake}) \times 100]$. The data were analyzed with ANOVA or *t*-test after an arcsine transformation as recommended by Kirk (1995).

Experiment 1: Rostral IC lesions and Flavor-nutrient preference learning

In Experiment 1 rats received lesions aimed at the rostral pole of the IC where gustatory and olfactory inputs converge on agranular insular neurons involved in flavor perception (Shipley & Geinisman, 1984; Cinelli et al., 1987). Flavor cues were paired with IG nutrient infusions during training. Two lesioned rats and one sham rat died during or after the surgeries and one sham rat became sick during training and was therefore discarded. Twelve rICx and seven sham rats completed this experiment.

Procedure

The CS solutions were cherry- and grape-flavored saccharin. The rats were given six one-bottle training sessions (30 min/day) with the CS+ solution paired with IG maltodextrin infusion (sessions 1, 3, 5) and the CS- solution paired with IG infusion of water (sessions 2, 4, 6). The right-left positions of the CS solutions varied following an ABBA sequence. A two-bottle preference test was then conducted with the CS+ vs. CS- for two 30 min/day sessions. During this test the rats were not infused and the left-right positions of the CS solutions were alternated from the first to the second session.

Results and discussion

Histology—Nissl staining of the brains slices from the twelve lesioned rats revealed bilateral lesions of the rostral portion of IC in seven rats, which formed the rICx group. A complete loss of neurons associated with glial proliferation and tissue collapse was observed (Figure 1A). The smallest and largest areas of lesion are illustrated in Figure 1B. The lesions extended from the ventral portion of the somatosensory cortex to the dorsal portions of the endopiriform nucleus and the piriform cortex. In most cases, the far lateral part of the granular subdivision of the IC was intact. In the rostrocaudal axis, the lesions extended from Frontal Plane 1.70 to Frontal Plane -0.30 of the atlas of Paxinos and Watson (1998).

The remaining five lesioned rats had incomplete lesions sparing most of the lateral part of the IC. The behavioral data from these five rats were discarded.

Behavior—Analysis of the one-bottle training data revealed that the CS intakes of the sham group were greater than those of rICx rats (11.6 vs. 8.4 g/30 min, $F(1, 12) = 9.4$, $p < 0.01$). Overall, the rats consumed more CS- than CS+ during training (10.8 vs. 9.2 g/30 min, $F(1, 12) = 16.0$, $p < 0.01$) probably due to the satiating effect of the IG maltodextrin infusions which limited CS+ intakes. The results of the two-bottle preference tests are summarized in Figure 2. Overall, the rats consumed more CS+ than CS-, $F(1, 12) = 52.4$; $p < 0.001$. The CS+ preferences of the rICx rats (90%) and sham rats (85%) did not differ significantly. As in training, the sham rats consumed more total fluid in the two-bottle test than did the rICx rats, $F(1, 12) = 7.5$, $p < 0.05$).

Experiment 2: Rostral IC lesions and Taste-nutrient preference learning

Experiment 1 showed that rats with lesions of the gustatory region of the IC learned to prefer the CS+ flavor that was paired with IG nutrient infusions. We previously reported that lesions of another taste relay, the medial PBN, blocked taste-nutrient but not flavor-nutrient learning, (Sclafani et al., 2001). The second experiment, therefore, investigated if gustatory IC lesions specifically interfere with nutrient-conditioned preferences when taste cues are used as CS. Supporting this possibility is the observation that inhibiting protein synthesis in the IC blocked taste aversion but not odor aversion conditioning in rats (Ferreira et al., 2005).

Procedure

The rICx and sham rats from Experiment 1 were trained with distinctive taste stimuli paired with IG maltodextrin (16%) and water infusions. The CS solutions contained 0.03% sucrose octaacetate + 0.2% saccharin (“bitter-sweet”) and 2% NaCl + 0.2% saccharin (“salty-sweet”). Compound CS solutions with a common sweet taste were used rather than single element CSs because food-deprived rats do not consume unsweetened bitter and salty solutions during 30-min sessions.

Results and discussion

During one-bottle training the rICx rats consumed less of the CS solutions than did sham rats (8.3 vs. 12.0 g/30 min, $F(1, 12) = 8.7$, $p < 0.05$). In the two-bottle preference test (Figure 3), the rICx rats continued to drink less than did the sham rats, $F(1, 12) = 10.4$, $p < 0.01$. However, both groups drank more CS+ than CS-, $F(1, 12) = 111.4$, $p < 0.001$. Furthermore, the percent CS+ intake of the rICx group exceeded that of the sham group (96% vs. 88%, $t(12) = 2.17$, $p < 0.05$).

The lesions of the rostral IC did not block taste preference learning induced by IG infusions of maltodextrin. The lesions damaged mainly the gustatory areas of the IC (boundaries between AP +3.0 mm and AP 0.0 mm according to Kosar et al., 1986), yet the rats were still able to associate taste as well as flavor cues with the viscerosensory stimuli generated by the carbohydrate infusions.

Experiment 3: Large IC lesions and Flavor-nutrient preference learning

Based on the finding that rats learned to prefer a flavor paired with electrical stimulation of the IC, Cubero and Puerto (2000) suggested that IC is critical in processing visceral stimuli, hedonic valence of the CS and/or food reward incentive learning. Given that lesions of the rostral pole of the IC failed to impair flavor preference learning (Exp. 1), the present experiment determined if large lesions of the IC which include both gustatory and viscerosensory regions would impair flavor-nutrient learning. Eighteen ICx and eight sham rats completed this experiment.

Procedure

The training and test procedures of Experiment 1 were used.

Results and discussion

Histology—Nissl staining of the brains slices from the eighteen lesioned rats revealed extensive and bilateral lesions of the IC in thirteen rats which formed the ICx group. A complete loss of neurons associated with glial proliferation was observed (Figure 4A). At the core of the lesion, a large cavity was noted probably due to tissue collapse. The smallest and largest areas of lesion are illustrated in Figure 4B. The lesions extended from the ventral portion of the somatosensory cortex to the rhinal sulcus. In most cases, the lesions encompassed the far lateral part of the caudate-putamen. Near total damage to the granular, dysgranular and agranular subdivisions of the IC was observed throughout its rostrocaudal extent (gustatory and visceral areas) from Frontal Plane 1.70 to Frontal Plane -2.80 of the atlas of Paxinos and Watson (1998).

The remaining five lesioned rats had incomplete lesions sparing most of the ventral part of the IC. The behavioral data from these five rats were discarded.

Behavior—Overall, the sham group consumed more CS solution during training than did the ICx group (12.2 vs. 9.3 g/30 min, $F(1, 19) = 5.3$, $p < 0.05$). Training intakes of the CS- exceeded that of CS+, (11.5 vs. 9.3 g/30 min, $F(1, 19) = 34.0$, $p < 0.001$). The ICx rats continued to drink less fluid compared to the sham rats in the two-bottle preference test $F(1, 19) = 5.3$, $p < 0.05$ (Figure 5). Both groups, however, consumed more CS+ than CS- in the choice test, $F(1, 19) = 176.8$; $p < 0.001$, and their CS+ preference were identical at 85%.

Experiment 4: Large IC lesions and Taste-nutrient learning

This experiment examined the ability of rats with large IC lesions to develop a preference for a taste CS+ that is paired with IG carbohydrate infusions over a CS- taste paired with IG water infusions.

Procedure

The rats from the third experiment were trained with bitter-sweet and salty-sweet CS solutions paired with IG carbohydrate and water infusions according to the procedures of Experiments 2.

Results and discussion

Analysis of the one-bottle training data revealed no difference in the CS intakes of the ICx and sham rats (8.7 vs. 9.9 g/30 min) or between the intakes of the CS+ and CS- solutions (8.8 vs. 9.5 g/30 min). In the two-bottle preference test (Figure 6), the sham and ICx rats consumed significantly more CS+ than CS- and their CS+ preferences did not differ significantly (sham: 90%; ICx: 82%). The groups also did not differ in their total CS intakes during the two-bottle test.

The IC lesions did not block taste-nutrient preference learning induced by IG infusions of maltodextrin. With large lesions that damaged both the gustatory and visceral areas of the IC, the rats were still able to process taste and visceral information, integrate them and form taste-visceral associations. Together with the results of Experiment 3, the findings indicate that large IC lesions had no effects on preference learning whether a complex flavor or taste was used as CS.

Experiment 5: Large IC lesions and Flavor-Taste preference learning

The previous four experiments investigated flavor and taste preference conditioning produced by the post-oral consequences of nutrients. Preferences and aversions can also be acquired based on an association between a CS flavor and an already preferred taste US (e.g., sweet) or unpreferred taste US (e.g., bitter) (Holman, 1975; Fanselow & Birk, 1982). Several studies indicate that flavor-taste learning and flavor-postingestive nutrient learning involve different processes (Holman, 1975; Giza et al., 1988; Elizalde & Sclafani, 1990; Myers & Hall, 1998). Recently, Sakai and Yamamoto (2001) reported that bilateral lesions of IC did not impair flavor-taste association learning in rats. In their study, thirsty rats were trained to drink one flavor mixed in a sweet saccharin solution and another flavor mixed in a bitter quinine solution. In a subsequent flavor-only choice test, the IC lesioned rats, like sham controls, selected the saccharin paired flavor. However, with this procedure it is not clear whether the choice was based on a specific preference for the sweet-paired flavor or an avoidance of the bitter-paired flavor (or both).

Experiment 5 addressed this issue by training ICx and sham rats with flavors mixed in highly preferred and less preferred sweet solutions. The CS+ and CS- training solutions both contained a Kool-Aid flavor mixed with 0.2% saccharin as in the prior experiments. However, the CS+ training solution also contained 8% fructose which made where as the CS- did not. This makes the CS+ solution more palatable than the CS- solution. Prior studies demonstrate that rats learn to prefer the fructose-paired CS+ solution over the CS- solution (Ackroff & Sclafani, 1991; Sclafani & Ackroff, 1994; Baker et al., 2003; Touzani & Sclafani, 2005). This conditioned preference appears to be due to the palatable taste of the fructose rather than to postingestive actions because IG fructose infusions do not condition a flavor preference in daily 30-min training sessions (Sclafani et al., 1993; Sclafani & Ackroff, 1994). Thus, under the conditions of the present experiment fructose-conditioned preferences represents a form of flavor-taste learning rather than flavor-postingestive consequence learning.

In addition to measuring the acquisition of the fructose-conditioned preference, Experiment 5 also measured the persistence of the preference during repeated extinction trials. This was of interest because Sakai & Yamamoto (2001) reported that IC lesions, unlike amygdala lesions, did not accelerate the extinction of the flavor preference. On the other hand, IC lesions are reported to facilitate extinction of conditioned taste aversions (Fresquet et al., 2004).

Procedure

The ICx and sham rats from Experiments 3 and 4 were trained during 30 min/day sessions with a CS+ solution containing 8% fructose solution (CS+/F) and a CS- solution without fructose. The CS flavors were orange-saccharin and lemon-lime-saccharin. The CS+/F was available on days 1, 3, 5 and 7; the CS- was available on days 2, 4, 6 and 8. During this training phase, CS intakes were limited to ~ 16 g /30 min session to prevent the animals from drinking substantially more of the CS+/F than the CS-. Two-bottle preference tests (30 min/day) were then conducted with the rats given unlimited access to the CS+ (without fructose) and CS- solutions. Two-bottle testing was extended for 16 daily sessions to determine if the persistence of the CS+ preference differed in the ICx and sham rats. The left-right position of the CS+ and CS- flavors alternated daily and the two-bottle data were expressed as 8 two-session means.

Results and discussion

During one-bottle training CS intakes were limited (to 16 g /30 min session) and there were no differences in the intakes of the ICx and sham rats (ICx: 14.7 g CS+, 14.3 g CS-; Sham: 15.2 g CS+, 15.3 g CS-). The results of the two-bottle tests are presented in Figure 7. To be consistent with the prior experiments, an analysis was first performed on the first two-bottle

test (mean of sessions 1 and 2). Overall, both groups consumed more CS+ than CS-, $F(1, 19) = 117.9$, $p < 0.001$ and there were no group effects. However, the ICx rats displayed a higher CS+ preference than did the controls (92% vs. 82%, $t(19) = 2.09$, $p < 0.05$). Within group comparisons revealed that over the 8 consecutive test blocks, CS+ intake but not CS- intake declined in the control group, CS x Test interaction, $F(7, 39) = 7.57$, $p < 0.01$; the controls consumed significantly more CS+ than CS- in tests 1 and 2 only. The ICx group showed a more gradual decline in CS+ intake with extinction, CS x Tests, $F(7, 84) = 5.86$, $p < 0.01$, and they consumed significantly more CS+ than CS- in tests 1-7 (test 8 $p < 0.06$). In the last test, the ICx rats displayed a higher CS+ preference, 80% vs. 60%, $t(19) = 2.09$, $p < 0.05$.

Both groups initially displayed a significant preference for the CS flavor that was mixed with fructose, confirming prior studies showing that the taste of fructose is an effective US (Baker et al., 2003; Baker et al., 2004; Touzani & Sclafani, 2005). The failure of IC lesions to impair flavor-taste preference learning confirms and extends the results of Sakai and Yamamoto (2001) obtained with rats trained with a saccharin-paired CS+ and quinine-paired CS- odor. In fact, the ICx rats in the present experiment displayed a stronger CS+ preference that persisted longer during extinction than did the control animals. Sakai and Yamamoto (2001) did not observe a more persistent CS+ preference in ICx rats in their study but their test sessions were shorter (5 vs. 30 min/session) and less extensive (8 vs. 16 sessions) than in the present experiment. Furthermore, in their one-trial training condition, the ICx tended to show a more persistent preference than did the controls.

GENERAL DISCUSSION

The aim of this study was to explore the role of the insular cortex in flavor preference learning produced by the post-oral or oral actions of nutrients. The results revealed that even large lesions of the insular cortex, that encompass the gustatory and visceral areas, did not prevent rats from learning to prefer a flavor paired with IG maltodextrin infusions or with the sweet taste of fructose.

Lesions of the insular cortex did not impair the ability of the rats to acquire an association between the orosensory stimuli and the viscerosensory information generated by intragastric infusions of carbohydrate (maltodextrin). The ICx rats, like their sham controls, learned to prefer the CS+ flavor or taste cues that were paired with the IG infusions of maltodextrin. In one experiment they displayed a stronger preference than did the controls (Exp. 2), whereas in the remaining three experiments the CS+ preferences of the ICx and control groups did not significantly differ. Insular cortex lesions also failed to prevent, and in fact enhanced the learning of a preference for a flavor that was mixed with fructose during training. Together, these findings show that the ability of rats with insular cortex lesions to process the orosensory information as well as the positive reinforcing signals generated by nutrients was not eliminated and indicate that the integrity of the insular cortex is not critical for flavor-nutrient and flavor-taste preference learning. Nevertheless, it is possible that IC lesions may have more subtle effects on flavor/taste preference learning that would be revealed with other behavioral test procedures.

Why IC lesions enhanced preference conditioning in some instances (Exp. 2 and 5) is not certain. The ICx rats consumed less CS during training than did the sham controls in some experiments (1, 2, 3) but this does not readily explain their increased CS+ preference. A post-hoc analysis of sham control data from Exp. 2 and 4 revealed that control animals that consumed the most ($n=5$) and least ($n=5$) amounts of CS during one-bottle training (13.8 vs. 10.4 g/30 min) displayed similar CS+ preferences (85.0 vs. 86.2%) in the two-bottle test. It is possible that preference conditioning was enhanced in the ICx rats because they perceived the CS+ and CS- flavors as more distinctive than did the sham controls. All CS solutions contained

saccharin, which may have resulted in some stimulus generalization between the CS+ and CS- flavors and taste mixtures. The insular cortex is implicated in the integration of gustatory and olfactory stimuli (Shipley & Geinisman, 1984; Cinelli et al., 1987) and IC lesions may reduce generalization between odor-taste and taste-taste mixtures.

The ICx rats displayed near-normal preference conditioning whether they were trained with complex flavor cues (Kool-Aid), which have distinctive odor components, or with taste mixtures. This contrasts with the selective disruptions in taste and flavor conditioning displayed by rats with medial PBN and amygdala lesions, respectively (Sclafani et al., 2001; Touzani & Sclafani, 2005). In addition, preference conditioning was not attenuated in rats with lesions limited to the gustatory IC as well as with rats with extensive lesions including both the gustatory and visceral IC. Although rats with only visceral IC lesions were not tested, it appears unlikely that this lesion would impair conditioning.

The failure of the IC lesions to impair flavor preference learning contrasts with the findings of Cubero and Puerto (2000) that insular cortex is involved in flavor preference learning. In their study, brain-intact rats learned to prefer a flavor paired with delayed electrical stimulation of the IC over another flavor that was not paired with brain stimulation. The present study taken together with this earlier work indicates that an intact IC is not needed for preference conditioning to occur although activation of local IC neurons can serve as a US to support such conditioning. One explanation of these findings is that stimulation of IC neurons activated other brain areas involved in processing visceral information generated by nutrients. The brain areas that relay and process the post-oral, nutrient US remain uncertain. The findings that amygdala and medial PBN lesions selectively impair flavor and taste conditioning, respectively, indicate that these lesions do not block processing of the visceral US (Sclafani et al., 2001; Touzani & Sclafani, 2005). Lesions of the lateral hypothalamus and area postrema also do not block the processing of the visceral US involved in flavor learning (Touzani & Sclafani, 2001; Touzani & Sclafani, 2002a; Touzani & Sclafani, 2002b). The lateral PBN has been suggested as a relay station for the visceral US relay based on the failure of lateral PBNx rats to develop a preference for a flavor mixed into 0.1 M sucrose (Reilly & Trifunovic, 2000). Preliminary work in our laboratory suggests that lateral PBN lesions do not block preference conditioning by IG nutrient infusions but this issue requires further study (Sclafani et al., 2003).

A number of lesion studies have investigated the role of the insular cortex in taste aversion learning with mixed results. Depending upon the lesion method, (neurotoxic vs. electrolytic), training procedure, and type of CS and US, IC lesions were observed to have no effect, attenuate or block aversion learning (e.g., Kiefer et al., 1982; Mackey et al., 1986; Kiefer & Morrow, 1991; Yamamoto et al., 1995; Cubero et al., 1999; Fresquet et al., 2004; Inui et al., 2006; Roman et al., 2006). In two studies in which IC lesions attenuated aversion conditioning, the ICx rats displayed more rapid extinction of the aversion than did control animals (Fresquet et al., 2004; Inui et al., 2006). In the last experiment of the present study, on the other hand, the ICx rats were more resistant than controls to extinction of the conditioned flavor preference. As previously noted, Sakai and Yamamoto (2001) found no difference between ICx and control rats in the extinction of a flavor-taste conditioned preference.

Why the ICx rats in the present study showed a more persistent taste-conditioned flavor preference is not certain. Balleine and Dickinson (2000) reported that in some circumstances, ICx rats were less affected than controls by manipulations that devalued the reward value of food stimuli. They hypothesized that the insular cortex is involved in encoding the incentive value of food instrumental outcomes. In the extinction procedure used in Experiment 5, the reward value of the CS+ was presumably devalued because the CS+ solution no longer contained the fructose solution. Thus, the persistent CS+ preference of the ICx rats may reflect

a deficit in their re-evaluation of the CS+ during the extinction trials. Since the percent CS+ preference in the very first choice test was higher in the ICx rats than in the control rats, it is also possible that ICx showed less extinction because they acquired a stronger preference during training. Consistent with this suggestion, we found that brain intact rats show more persistent fructose-conditioned CS+ preferences when the amount of CS solutions available during training sessions was increased (Yiin et al., 2005).

The Balleine and Dickinson (2000) incentive memory hypothesis cited above suggests that an instrumental test of conditioned flavor preferences may reveal an IC lesion deficit not observed in the two-bottle consumption tests used in the present study. Normal rats trained to make different instrumental responses to obtain flavored solutions in an operant choice situation would be expected to make more responses for a flavored solution (CS+) after it had been paired with IG nutrient infusions. IC lesion animals should show a similar instrumental preference in a reinforced test that allowed them to taste the CS solutions, i.e., an instrumental consumption test. However, they may show a preference deficit, relative to controls, in an instrumental extinction test where the CS solutions are not available to activate their incentive memory.

Another approach to study the role of the insular cortex in taste learning involves temporary rather than permanent disruptions in neural function. A number of studies indicate that temporary inactivation of IC function using local infusion of a protein synthesis inhibitor or neurotransmitter antagonists disrupts taste aversion learning (Rosenblum et al., 1993; see Bermudez-Rattoni, 2004). IC inactivation is also reported to disrupt learned taste safety, i.e., the attenuation of a neophobic response to a novel tastant (e.g., saccharin) (Bermudez-Rattoni, 2004; Figueroa-Guzman et al., 2006; but see Ferreira et al., 2005). In view of these results, it is possible that temporary inactivation of the IC may impair flavor (or taste) preference learning conditioned by nutrients. If so, this would indicate that the preference conditioning observed in IC lesioned animals represents functional recovery and/or reorganization of the neural systems involved in flavor-nutrient learning.

The failure of IC lesions to disrupt flavor preference conditioning are relevant to the recent suggestion that the insular cortex is critical for behaviors “whose bodily effects become pleasurable through learning” (Naqvi et al., 2007). The idea was based on the clinical finding that humans with IC damage lost their addiction to cigarette smoking but apparently did not alter their desire for food. While the authors suggest that the bodily effects of eating are inherently pleasurable, clearly learning has an important role in the development of food preferences in animals and humans (Capaldi, 1996; Sclafani, 1999; Yeomans, 2006). The present findings indicate that, in rats at least, such learned preferences are not dependent upon an intact insular cortex. This does not, however, preclude a role for the insular cortex in the learned pleasures (or hedonic evaluation) of food since some data indicate that nutrient conditioning may enhance flavor preferences without necessarily increasing the flavor hedonics (Myers & Sclafani, 2003).

Acknowledgments

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-31135. We thank Dr. Karen Ackroff for her helpful comments and anonymous reviewers for insightful interpretations that were incorporated into this paper.

Abbreviations

AMY, amygdala; CTA, conditioned taste aversion; IC, insular cortex; ICx, insular cortex lesions; CS, conditioned stimulus; IG, intragastric; PBN, parabrachial nucleus; rICx, rostral insular cortex lesion; US, unconditioned stimulus.

References

- Ackroff K, Sclafani A. Flavor preferences conditioned by sugars: Rats learn to prefer glucose over fructose. *Physiol.Behav* 1991;50:815–824. [PubMed: 1775557]
- Baker BJ, Booth DA. Preference conditioning by concurrent diets with delayed proportional reinforcement. *Physiol.Behav* 1989;46:585–590. [PubMed: 2602482]
- Baker RM, Li Y, Lee M, Sclafani A, Bodnar RJ. Naltrexone does not prevent acquisition or expression of flavor preferences conditioned by fructose in rats. *Pharmacol.Biochem.Behav* 2004;78:239–246. [PubMed: 15219763]
- Baker RM, Shah MJ, Sclafani A, Bodnar RJ. Dopamine D1 and D2 antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats. *Pharmacol.Biochem.Behav* 2003;75:55–65. [PubMed: 12759113]
- Balleine BW, Dickinson A. The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. *J.Neurosci* 2000;20:8954–8964. [PubMed: 11102506]
- Bermudez-Rattoni F. Molecular mechanisms of taste-recognition memory. *Nat. Rev. Neurosci* 2004;5:209–217. [PubMed: 14976520]
- Bermúdez-Rattoni F, Rusiniak KW, Garcia J. Flavor-illness aversions: potentiation of odor by taste is disrupted by application of novocaine into amygdala. *Behav.Neural Biol* 1983;37:61–75. [PubMed: 6309134]
- Bures, J.; Bermúdez-Rattoni, F.; Yamamoto, T. *Conditioned Taste Aversion: Memory of a Special Kind*. Oxford University Press; Oxford: 1998.
- Capaldi, ED. Conditioned food preferences. In: Capaldi, ED., editor. *Why We Eat What We Eat: The Psychology of Eating*. American Psychological Association; Washington, DC: 1996. p. 53-80.
- Cinelli AR, Ferreyra-Moyano H, Barragan E. Reciprocal functional connections of the olfactory bulbs and other olfactory related areas with the prefrontal cortex. *Brain Res. Bull* 1987;19:651–661. [PubMed: 3440217]
- Cubero I, Puerto A. Electrical stimulation of the insular cortex induces flavor-preferences. *Brain Res* 2000;872:134–140. [PubMed: 10924685]
- Cubero I, Thiele TE, Bernstein IL. Insular cortex lesions and taste aversion learning: effects of conditioning method and timing of lesion. *Brain Res* 1999;839:323–330. [PubMed: 10519056]
- Dunn LT, Everitt BJ. Double dissociations 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. *Behav.Neurosci* 1988;102:3–23. [PubMed: 3281693]
- Elizalde G, Sclafani A. Flavor preferences conditioned by intragastric Polycose infusions: A detailed analysis using an electronic esophagus preparation. *Physiol.Behav* 1990;47:63–77. [PubMed: 2109327]
- Ettenberg A, White N. Conditioned taste preferences in the rat induced by self-stimulation. *Physiol.Behav* 1978;21:363–368. [PubMed: 740753]
- Fanselow MS, Birk J. Flavor-flavor associations induce hedonic shifts in taste preference. *Anim.Learn.Behav* 1982;10:223–228.
- Ferreira, G.; Ferry, B.; Ravel, N.; Levy, F. Program No. 71.13. 2005 Abstract Viewer/Itinerary Planner. Society for Neuroscience; Washington, DC: 2005. 2005. Protein synthesis in the insular cortex is necessary for aversive taste memory formation but neither for aversive odor nor for appetitive taste memory in rats. Online
- Figueroa-Guzman Y, Kuo JS, Reilly S. NMDA receptor antagonist MK-801 infused into the insular cortex prevents the attenuation of gustatory neophobia in rats. *Brain Res* 2006;1114:183–186. [PubMed: 16935274]
- Fresquet N, Angst MJ, Sandner G. Insular cortex lesions alter conditioned taste avoidance in rats differentially when using two methods of sucrose delivery. *Behav.Brain Res* 2004;153:357–365. [PubMed: 15265630]
- Fu W, Sugai T, Yoshimura H, Onoda N. Convergence of olfactory and gustatory connections onto the endopiriform nucleus in the rat. *Neuroscience* 2004;126:1033–1041. [PubMed: 15207336]
- Giza BK, Antonucci RF, Sclafani A, Scott TR. Gustatory neural responses to polysaccharide and starch in the rat. *Soc.Neurosci.Abstr* 1988;14

- Hanamori T, Kunitake T, Kato K, Kannan H. Responses of neurons in the insular cortex to gustatory, visceral, and nociceptive stimuli in rats. *J. Neurophysiol* 1998;79:2535–2545. [PubMed: 9582226]
- Holman EW. Immediate and delayed reinforcers for flavor preferences in the rat. *Learn. Motiv* 1975;6:91–100.
- Inui T, Shimura T, Yamamoto T. Effects of brain lesions on taste-potentiated odor aversion in rats. *Behav. Neurosci* 2006;120:590–599. [PubMed: 16768611]
- Kiefer SW, Morrow NS. Odor cue mediation of alcohol aversion learning in rats lacking gustatory neocortex. *Behav. Neurosci* 1991;105:25–32. [PubMed: 1851018]
- Kiefer SW, Rusiniak KW, Garcia J. Flavor-illness aversions: gustatory neocortex ablations disrupt taste but not taste-potentiated odor cues. *J. Comp. Physiol. Psychol* 1982;96:540–548. [PubMed: 6288779]
- Kirk, RE. *Experimental Design: Procedures for the Behavioral Sciences*. Brooks/Cole; Pacific Grove, CA.: 1995.
- Kosar E, Grill HJ, Norgren R. Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. *Brain Res* 1986;379:329–341. [PubMed: 3742225]
- Mackey WB, Keller J, van der Kooy D. Visceral cortex lesions block conditioned taste aversions induced by morphine. *Pharmacol. Biochem. Behav* 1986;24:71–78. [PubMed: 3945668]
- Myers KP, Hall WG. Evidence that oral and nutrient reinforcers differentially condition appetitive and consummatory responses to flavors. *Physiol. Behav* 1998;64:493–500. [PubMed: 9761223]
- Myers KP, Sclafani A. Conditioned acceptance and preference but not altered taste reactivity responses to bitter and sour flavors paired with intragastric glucose infusion. *Physiol. Behav* 2003;78:173–183. [PubMed: 12576114]
- Nachman M, Ashe JH. Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. *J. Comp. Physiol. Psychol* 1974;87:622–643. [PubMed: 4426986]
- Naqvi NH, Rudrauf D, Damasio H, Bechara A. Damage to the insula disrupts addiction to cigarette smoking. *Science* 2007;315:531–534. [PubMed: 17255515]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press; San Diego, CA.: 1998.
- Pérez C, Fanizza LJ, Sclafani A. Flavor preferences conditioned by intragastric nutrients in rats fed chow or a cafeteria diet. *Appetite* 1999;32:155–170. [PubMed: 9989925]
- Reilly S, Trifunovic R. Lateral parabrachial nucleus lesions in the rat: aversive and appetitive gustatory conditioning. *Brain Res. Bull* 2000;52:269–278. [PubMed: 10856824]
- Roman C, Nebieridze N, Sastre A, Reilly S. Effects of lesions of the bed nucleus of the stria terminalis, lateral hypothalamus, or insular cortex on conditioned taste aversion and conditioned odor aversion. *Behav. Neurosci* 2006;120:1257–1267. [PubMed: 17201470]
- Rosenblum K, Meiri N, Dudai Y. Taste memory: the role of protein synthesis in gustatory cortex. *Behav. Neural Biol* 1993;59:49–56. [PubMed: 8442732]
- Sakai N, Yamamoto T. Possible routes of visceral information in the rat brain in formation of conditioned taste aversion. *Neurosci. Res* 1999;35:53–61. [PubMed: 10555164]
- Sakai N, Yamamoto T. Effects of excitotoxic brain lesions on taste-mediated odor learning in the rat. *Neurobiol. Learn. Memory* 2001;75:128–139.
- Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol* 1982;210:163–173. [PubMed: 7130477]
- Scalera G. Effects of conditioned food aversions on nutritional behavior in humans. *Nutr. Neurosci* 2002;5:159–188. [PubMed: 12041874]
- Sclafani, A. Macronutrient-conditioned flavor preferences. In: Berthoud, H-R.; Seeley, R., editors. *Neural and metabolic control of macronutrient intake*. CRC Press; Boca Raton, FL: 1999. p. 93-107.
- Sclafani A, Ackroff K. Glucose- and fructose-conditioned flavor preferences in rats: Taste versus postingestive conditioning. *Physiol. Behav* 1994;56:399–405. [PubMed: 7938256]
- Sclafani, A.; Ackroff, K.; Norgren, R. Program No. 508.8. 2003 Abstract Viewer/Itinerary Planner. Society for Neuroscience; Washington, DC: 2003. 2003. Lateral parabrachial nucleus (LPBN) lesions fail to block flavor-nutrient preference learning in rats. Online
- Sclafani A, Azzara AV, Touzani K, Grigson PS, Norgren R. Parabrachial nucleus lesions block taste and attenuate flavor preference and aversion conditioning in rats. *Behav. Neurosci* 2001;115:920–933. [PubMed: 11508731]

- Sclafani A, Cardieri C, Tucker K, Blusk D, Ackroff K. Intragastric glucose but not fructose conditions robust flavor preferences in rats. *Am.J.Physiol* 1993;265:R320–R325. [PubMed: 8368385]
- Shipley MT, Geinisman Y. Anatomical evidence for convergence of olfactory, gustatory, and visceral afferent pathways in mouse cerebral cortex. *Brain Res. Bull* 1984;12:221–226. [PubMed: 6722597]
- Touzani K, Sclafani A. Conditioned flavor preference and aversion: Role of the lateral hypothalamus. *Behav.Neurosci* 2001;115:84–93. [PubMed: 11256455]
- Touzani K, Sclafani A. Area postrema lesions impair flavor-toxin aversion learning but not flavor-nutrient preference learning. *Behav.Neurosci* 2002a;116:256–266. [PubMed: 11996311]
- Touzani K, Sclafani A. Lateral hypothalamic lesions impair flavor-nutrient and flavor-toxin trace learning in rats. *Eur.J.Neurosci* 2002b;16:2425–2433. [PubMed: 12492437]
- Touzani K, Sclafani A. Critical role of amygdala in flavor but not taste preference learning in rats. *Eur.J.Neurosci* 2005;22:1767–1774. [PubMed: 16197517]
- Yamamoto T, Fujimoto Y, Shimura T, Sakai N. Conditioned taste aversion in rats with excitotoxic brain lesions. *Neurosci.Res* 1995;22:31–49. [PubMed: 7792081]
- Yamamoto T, Shimura T, Sako N, Yasoshima Y, Sakai N. Neural substrates for conditioned taste aversion in the rat. *Behav.Brain Res* 1994;65:123–127. [PubMed: 7718144]
- Yeomans, MR. The role of learning in development of food preferences. In: Shepherd, R.; Raats, M., editors. *Psychology of food choice*. CBI; Oxfordshire: 2006. p. 93-112.
- Yiin Y-M, Dwyer D, Sclafani A. Conditioning and extinction of flavor-flavor and flavor-nutrient preferences in rats. *Appetite* 2005;44:391.

Figure 1.

Schematic representation of the extent of the smallest (shaded area) and largest (crosshatched area) insular cortex lesions at four rostrocaudal levels. The numbers beside each plate indicate the distance anterior or posterior to bregma. The plates are adapted from the atlas of Paxinos and Watson (1998) with permission.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 2.

Experiment 1. Effects of rostral insular cortex lesions (rICx, n=7) and Sham lesions (n=7) on intakes of the CS+ and CS- flavor solutions during two-bottle preference test sessions. During the one-bottle training, the CS+ was paired with IG infusions of 16% maltodextrin and the CS- with IG water infusions. Numbers atop bars represent mean percentage intakes of CS+. Data are represented as mean + SEM.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 3.

Experiment 2. Effects of rostral insular cortex lesions (rICx, n=7) and Sham lesions (n=7) on intakes of the CS+ and CS- taste solutions during two-bottle preference test sessions. During the one-bottle training, intakes of the CS+ and CS- were paired with IG infusions of 16% maltodextrin and water, respectively. Numbers atop bars represent mean percentage intakes of CS+. Data are represented as mean + SEM.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 4.

Schematic representation of the extent of the smallest (shaded area) and largest (crosshatched area) insular cortex lesions at seven rostrocaudal levels. The numbers beside each plate indicate the distance anterior or posterior to bregma. The plates are adapted from the atlas of Paxinos and Watson (1998) with permission.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 5.

Experiment 3. Effects of large insular cortex lesions (ICx, n=13) and Sham lesions (n=8) on intakes of the CS+ and CS- flavor solutions during two-bottle preference test sessions. During the one-bottle training, the CS+ was paired with IG infusions of 16% maltodextrin and the CS- with IG water infusions. Numbers atop bars represent mean percentage intakes of CS+. Data are represented as mean + SEM.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 6.

Experiment 4. Effects of large insular cortex lesions (ICx, n=13) and Sham lesions (n=8) on intakes of the CS+ and CS- taste solutions during two-bottle preference test sessions. During the one-bottle training, intakes of the CS+ and CS- were paired with IG infusions of 16% maltodextrin and water, respectively. Numbers atop bars represent mean percentage intakes of CS+. Data are represented as mean + SEM.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats

Figure 7.

Experiment 5. Effects of large insular cortex lesions (ICx, n=13) and Sham lesions (n=8) on intakes of the CS+ and CS- flavor solutions during two-bottle preference test sessions. During one-bottle training, the CS+ solution contained 8% fructose and CS- did not contain fructose. In the two-bottle tests, neither CS solutions contained sugar. Numbers atop bars represent mean percentage intakes of CS+. Data are represented as mean + SEM.

Touzani & Sclafani (2007) Insular cortex lesions failed to block flavor and taste preference learning in rats