Published in final edited form as:
Brain Res. 2015 March 2; 1599: 9–19. doi:10.1016/j.brainres.2014.12.035.

Unconditioned Oromotor Taste Reactivity Elicited by Sucrose and Quinine is Unaffected by Extensive Bilateral Damage to the Gustatory Zone of the Insular Cortex in Rats

Camille Tessitore King[a], **Koji Hashimoto**[b,c], **Ginger D. Blonde**[c], and **Alan C. Spector**[c]

Camille Tessitore King: cking@stetson.edu; Koji Hashimoto: coji@kumamoto-u.ac.jp; Alan C. Spector: spector@psy.fsu.edu

[a]Department of Psychology, Stetson University, DeLand, FL, USA

[b]Department of Morphological and Physiological Sciences, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

[c]Department of Psychology & Program in Neuroscience, Florida State University, Tallahassee, FL

Abstract

Rats display stereotypical oromotor and somatic responses to small volumes of intraorally infused taste solutions. These behaviors, known as taste reactivity, are categorized by their association with ingestion or rejection and are thought to reflect the palatability of the stimulus. Because supracollicular decerebrate rats display normal taste reactivity responses, it would appear that forebrain structures are not necessary for generating them. However, because moving the plane of transection rostrally, or damaging or manipulating specific ventral forebrain sites disrupts normal taste reactivity behavior, lesions of the gustatory cortex, a region that has been suggested to be involved with palatability processing, may do the same. In the current study, rats received two injections of either ibotenic acid (N=12) or vehicle (N=8), targeting the conventionally defined gustatory cortex in each hemisphere, and were implanted with intraoral cannulae. Following recovery, their responses to intraoral infusions (0.23 ml in 1 min) of dH_20, sucrose (1.0M and 0.1M), and quinine hydrochloride (3 mM and 0.3 mM) were video recorded. Analysis of brains with sufficient bilateral lesions (N=10) revealed that, on average, approximately 94% of the gustatory cortex was destroyed. These extensive bilateral lesions had no significant effect on taste reactivity; the numbers of ingestive and aversive responses to sucrose and quinine were similar between groups. Though these findings do not rule out involvement of the gustatory cortex in palatability processing, they make evident that the region of insular cortex destroyed is not necessary for the normal expression of unconditioned affective behavioral responses to taste stimuli.

Corresponding Author: Camille Tessitore King, Ph.D., Department of Psychology, 421 N. Woodland Blvd., Unit 8281, Stetson University, DeLand, FL 32723, Office phone: 386-822-7093.

Keywords

cortex; motivation; hedonic; palatability; taste reactivity

1. Introduction

The behavioral responses a taste stimulus elicits can be divided into two functional subclasses: appetitive and consummatory (see Berridge, 2000; Spector, 2000). Craig (1918) described appetitive responses as those behaviors which bring the animal into contact with a taste stimulus (e.g., foraging, approaching a drinking spout) and consummatory responses as reflex-like actions that are elicited once a taste stimulus makes contact with oral sensory receptors (e.g., swallowing, oromotor responses). One- and two-bottle intake tests, commonly used assays of taste palatability, involve both appetitive and consummatory behavior because the animal must approach and make contact with the licking spout (the appetitive components) upon which oromotor responses (the consummatory component) are elicited by the stimulus once it engages the receptors of the oral cavity. Purely consummatory responses can be measured via the delivery of taste solutions through a surgically implanted intraoral cannula (Berridge, 1996; Grill and Norgren, 1978a; Grill et al., 1987). When taste stimuli are delivered in this way, rats elicit stereotypical affective behavioral responses referred to as taste reactivity (TR, Grill and Norgren, 1978a). These unconditioned reflex-like behaviors are thought to reflect the palatability evaluation of taste stimuli (Berridge, 2000; Grill and Berridge, 1985). Normally preferred stimuli (e.g., sucrose) elicit responses that promote ingestion (i.e., ingestive behaviors) while taste stimuli that are normally avoided (e.g., quinine) elicit responses that promote rejection (i.e., aversive behaviors; Grill and Norgren, 1978a).

Some years ago, Pfaffmann et al. (1977) speculated that taste hedonics were mediated by the ventral forebrain pathway that arises from the gustatory zone of the parabrachial nucleus (PBN), whereas sensory-discriminative functions (e.g. qualitative identification) were associated with the gustatory thalamocortical pathway. The extent of forebrain involvement in affective behavioral responses, specifically TR responses, to taste stimuli however was seriously challenged by Grill and Norgren (1978b) by demonstrating that chronic supracollicular decerebrate rats were able to elicit TR responses to sucrose and quinine that did not differ from intact controls. In this preparation, only two gustatory relays in the brainstem, the nucleus of the solitary tract (NST) and the PBN, remain neurally connected to motor output circuits. Although this finding precludes the necessity of the forebrain in triggering unconditioned affective oromotor and somatic behaviors, several lines of investigation have demonstrated forebrain control over them. For instance, when Grill and Norgren (1978b) moved their plane of transection rostrally to just anterior of the thalamus, rats displayed enhanced aversive TR behaviors to all taste stimuli. In fact, in the chronic thalamic preparation, TR responses associated with ingestion were completely absent. These findings indicated that neural mechanisms rostral to the midbrain somehow modulate the hedonic impact of a taste stimulus. Candidate forebrain sites for such affective processing of taste input include the central nucleus of the amygdala (Touzani et al., 1997) and the ventral pallidum/substantia innominata (Cromwell and Berridge, 1993) as localized destruction of

these structures leads to increases in the aversive impact of taste stimuli, findings that buttress Pfaffmann's (1977) hypothesis that the ventral forebrain pathway stemming from the PBN is involved with the mediation of taste hedonics. Moreover, pharmacological manipulations of ventral forebrain structures have provided further support for their role in affective taste processing. Infusions of mu opioid agonists into the ventral pallidum or nucleus accumbens, for example, have been shown to enhance ingestive responses to oral infusions of sucrose and attenuate aversive responses to oral infusions of quinine (Peciña and Berridge, 2005; Peciña et al., 2006; Smith and Berridge, 2005, 2007). The results from the aforementioned studies highlight the fact that the lack of an effect of a neural insult at lower levels of the ascending gustatory system (i.e., the decerebrate rat preparation) is not necessarily emulated by more targeted manipulations (pharmacological or lesions) at higher levels.

Clearly, the ventral forebrain plays a role in affective taste processing, but evidence for the gustatory cortex (GC), too, has also been accumulating. In fact, very early research showed elevations in quinine avoidance thresholds in rats with large ablations of the GC (Benjamin 1955a, 1955b; Benjamin and Akert, 1959). Much more recently, based on electrophysiological recordings from awake rats presented with intraorally delivered taste stimuli, some investigators have suggested that GC neurons are involved in processing the palatability of taste stimuli (e.g., Grossman et al., 2008; Katz et al., 2001; Sadacca et al., 2012). That said, relatively normal preference-avoidance functions for a variety of taste stimuli measured by intake tests in rats with substantial GC damage have been demonstrated (Benjamin 1955a, 1955b; Braun et al., 1982; Dunn and Everitt, 1988). Thus, the necessity of the GC for expression of taste affect remains unclear. Intake tests, however, can be influenced by other factors such as postingestive events, thereby providing only a partial analysis of affective gustatory responsiveness. To overcome this limitation, Hashimoto and Spector (2014) employed a brief-access taste test, which minimizes postingestive factors (e.g., Davis, 1973; Smith, 2001; Spector, 2003), to assess the effects of GC lesions on unconditioned licking to sucrose and quinine. Still, no differences were observed between the animals with and without GC lesions, suggesting that the damaged region of the GC was unnecessary for the normal expression of taste-triggered unconditioned licking responses and their suppression. However, brief-access taste tests (as well as intake tests) rely in part on appetitive behavior because they require the animal to voluntarily approach the drinking spout. Moreover, the rats in the Hashimoto and Spector (2014) study were either food- or water-deprived during testing to promote stimulus sampling, and this may have modulated the responsiveness of the animals.

The TR procedure, on the other hand, is well suited to test taste-related palatability unencumbered by postingestive stimulation and physiological need states. Because in this paradigm the stimulus is directly infused into the oral cavity under explicit experimenter control, no appetitive behavior is involved and thus it represents a relatively pure assessment of consummatory responsiveness. In addition, the infusion of a very small volume of the taste stimulus under study over a relatively brief period of time obviates the contribution of postingestive events to the responses observed. Thus, in the current study, to further examine the role of the GC in palatability processing in the rat model, we tested whether large neurotoxic lesions of the GC would disrupt unconditioned TR to two prototypical

tastants: sucrose, a normally preferred stimulus that is sweet to humans and potently elicits ingestive oromotor behavior, and quinine, a normally avoided stimulus that is bitter to humans and potently elicits aversive oromotor behavior.

2. Results

2.1. Lesion analysis

Our objective was to produce extensive bilateral damage to the conventionally defined GC, which we accomplished in 10 out of the 12 rats that received ibotenic acid injections targeting the GC (GCX). Fig. 1 depicts the region of insular cortex targeted and Fig. 2 provides an example of one case that met the criteria for a successful lesion. In the brain illustrated, 97.28% of the GC and 100% of its core region was destroyed bilaterally (Figs. 2 and 3). Such extensive GC damage was typically observed in the rats meeting the histological inclusion criteria. In fact, on average, 93.97 ± 0.02 % of the GC and 97.75 % ± 0.01 of the GC core was damaged in these rats (Fig. 4). Clearly, the lesions were large, as intended, and sometimes encroached upon brain regions surrounding the GC including the claustrum, the granular layer of insular cortex, and some portions of insular cortex both anterior and posterior to GC. The two GCX rats with incomplete lesions were excluded from the data analysis resulting in a GCX sample size of N=10.

2.2. Taste Reactivity Analysis

2.2.1. Quinine reactivity—Nearly complete bilateral lesions of the GC did not impair unconditioned TR responses to quinine (Fig. 5). A 2-way repeated measures ANOVA (lesion x quinine concentration) revealed no significant effect of the lesion on Total Ingestive Score ($F_{1,15}$ = 1.22, P = 0.29). A concentration effect ($F_{1,15}$ = 12.43, P = 0.003), but no interaction (P = 0.70), was observed with more ingestive behaviors occurring at the lower concentration of quinine for both groups. The Total Aversive Score for both SHAM and GCX groups was also similar ($F_{1,15}$ = 1.23, P = 0.29). Again, a concentration effect ($F_{1,15}$ = 148.26, P < 0.00001) was observed, with fewer aversive behaviors being elicited by the lower concentration of quinine. No interaction was revealed (P = 0.93). Refer to Tables 1 and 2 for the mean numbers of individual TR responses elicited by the intraoral infusions of quinine.

The latency to the first gape for SHAM and GCX animals was 14.71 ± 3.83 and 12.44 ± 5.16 s, respectively, at the lower quinine concentration and was 2.14 ± 1.14 and 2.70 ± 0.93 s, respectively, at the higher concentration. Two sample t-tests showed no significant differences in this measure between the groups at either the 0.0003M (P = 0.73) or the 0.003M (P = 0.71) concentration of quinine.

2.2.2. Sucrose reactivity—Unconditioned TR responses to sucrose were also unimpaired by the extensive bilateral lesions of the GC (Fig. 6). A 2-way repeated measures ANOVA (lesion x sucrose concentration) showed no significant effect of the lesion on Total Ingestive Score ($F_{1,15}$ = 0.002, P = 0.97). Neither a concentration effect ($F_{1,15}$ = 0.09, P = 0.78) nor interaction (P = 0.84) was noted. Likewise, for Total Aversive Score, there was no effect of the lesion ($F_{1,15}$ = 0.19, P = 0.67), no concentration effect ($F_{1,15}$ = 0.07, P = 0.79) and no

interaction ($P = 0.13$). Tables 1 and 2 list the mean TR responses elicited by the intraoral infusions of sucrose.

2.2.3. Water reactivity—A two-sample t-test revealed a significantly higher ($P = 0.03$) Total Ingestive Score in response to the intraoral delivery of dH_2O for the SHAM group (M = 70.86 ± 10.00) as compared with the GCX group (M = 38.60 ± 8.46) on the final habituation day of the first week of testing. This mean difference, however, was eliminated ($P = 0.69$) by the final habituation day of the second week of testing (SHAM, M = 58.00 ± 8.65; GCX, M = 62.50 ± 6.94). When the averages of these two water scores for each group (SHAM, 64.43 ± 6.65; GCX, 50.55 ± 6.26) were compared, no significant difference between groups ($P = 0.15$) was observed. Bonferroni corrected paired t-tests revealed that the Total Ingestive Score for each concentration of sucrose was significantly different from water for each group (all p-values < 0.03) with one exception. For the SHAM group, the comparison between 0.1M sucrose and water approached significance ($P = 0.06$). The average scores for each TR behavior in response to water are listed in Tables 1 and 2 and graphed in Figs. 5 and 6. There was virtually no aversive behavior elicited by water.

3. Discussion

The extensive region of GC damaged bilaterally in these rats is not necessary for the production of unconditioned affective oromotor and somatic responses to sucrose and quinine. The numbers of both ingestive and aversive TR responses were quite similar between GCX and SHAM rats at the two concentrations of each tastant tested. Although SHAM rats did elicit significantly more ingestive responses to dH_2O initially, the difference was attenuated when the rats were tested with dH_2O a second time a few weeks later. It was somewhat surprising that there was no effect of sucrose concentration on ingestive TR, perhaps related to the order of stimulus testing, but the sucrose ingestive scores for both concentrations were nonetheless statistically different than those for water (P's < 0.03) or approached a statistically significant difference ($P = 0.06$), and importantly, did not differ between surgical groups.

The failure of these large lesions to affect oromotor and somatic affective responses is consistent with previous research showing a lack of an effect of GC lesions on unconditioned taste preferences and aversions as measured in single-bottle or two-bottle intake tests (Benjamin 1955a, 1955b; Braun et al., 1982; Dunn and Everitt, 1988) or in brief-access taste tests (Hashimoto and Spector, 2014). The fact that the TR procedure substantially minimizes postingestive consequences and does not require manipulation of physiological need states strongly suggests that the measured behaviors were taste-guided and strengthens the conclusion that the GC, as traditionally defined, is not necessary for normal hedonic evaluation of taste stimuli.

In contrast to our lack of an effect of GC lesions on TR responsiveness to quinine, others have reported elevated quinine avoidance thresholds in 48-h two-bottle tests (Benjamin, 1955a, 1955b; Benjamin and Akert, 1959; Benjamin and Pfaffmann, 1955) in cortically ablated rats. Accordingly, one might argue that cortical tissue spared by the lesions in our study may have been responsible for maintaining TR responsiveness. Because the lesions in

our study destroyed, on average, ~94% of GC and ~98% of its core region, it is improbable that the small amount of spared tissue *within* GC was responsible for maintaining normal TR. However, in light of recent findings concerning the functional topography of insular cortex, it is reasonable to speculate that cortical tissue *outside* the conventionally defined boundaries of GC may have been sufficient to support normal TR responsiveness. Using the same high-resolution lesion mapping program described here (in *4.5*, below), Schier et al. (2014) reported that while ibotenic acid lesions of the traditionally defined GC did not impair the learning or retention of a CTA, neural damage in more posterior regions of insular cortex, coupled with posterior GC damage, was associated with CTA deficits. In a similar fashion, ablation of these posterior insular cortical regions may lead to the disruption of TR and other unconditioned taste preferences and aversions. Targeting these regions with smaller, more selective lesions would be instructive in this regard.

The current findings do not necessarily mean that neural activity in the GC does not contribute to the processing of taste signals in a manner related to the affective behaviors measured. As an example, using the TR procedure in combination with Fos immunohistochemistry, we recently observed more quinine- than water-stimulated neural activity throughout the rostrocaudal extent of the GC (King et al., 2014). That transection of both the glossopharyngeal nerve and the chorda tympani nerve attenuated the numbers of quinine-stimulated Fos-neurons and their regeneration restored them confirmed that the neural response was taste-mediated and not due to postingestive stimulation (King et al., 2014). Of particular relevance to the current study, significant correlations ($r = 0.65$ to $r = .75$) between the numbers of quinine-stimulated gapes and Fos positive neurons in regions approximating DI and AI throughout GC were found. Moreover, numerous electrophysiological studies in the rat (Allen et al., 1991; Cechetto and Saper, 1987; de Araujo and Simon, 2009; Hanamori et al., 1998; Katz et al., 2001, 2002; Kosar et al., 1986a; Norgren and Wolf, 1975; Sadacca et al., 2012; Saper, 1982; Yamamoto et al, 1980, 1985, 1989), as well as optical imaging studies in the rat (Accolla and Carleton, 2008; Accolla et al., 2007,) and mouse (Chen et al., 2011), and functional magnetic resonance imaging in the rat (Kida et al., 2011) have also shown that the GC, as traditionally defined, contains neurons that respond to various features of taste stimuli, possibly including their hedonic impact. Katz and colleagues (Sadacca et al., 2012), for instance, contend that palatability processing in the GC actually occurs in advance of palatability processing in the amygdala, and possibly in advance of brainstem neural activity associated with TR behaviors (Travers and Norgren, 1986). In all of these cases, neuronal responses are correlated with various features of the tastant, providing insight into the organization of gustatory information in insular cortex; however, such data do not explicitly reveal the function to which the stimulus-related activity contributes.

Whatever functional role the GC is playing in palatability processing, the results presented here provide compelling evidence that this portion of the gustatory system is unnecessary for normal TR to be expressed, at least to sucrose and quinine, by rats. In other words, with respect to TR behavior, rats with extensive damage to the conventionally defined GC functionally resemble supracollicular decerebrate rats more than they resemble rats with a more rostral plane of brain transection (i.e., rostral to the thalamus) or rats with lesions to or

pharmacological manipulations of ventral forebrain sites such as the ventral pallidum, amygdala, and nucleus accumbens. Lesions targeting neurons in the parvicellular portion of the ventroposteromedial nucleus of thalamus (VPMpc; i.e., the gustatory thalamus), the axons of which project to GC, do not affect TR to sucrose, NaCl or HCl, but do paradoxically increase both ingestive and aversive TR to a high concentration of quinine (Flynn et al., 1991). On the whole, it would appear that, just as Pfaffmann et al. (1977) speculated, the ventral forebrain gustatory pathway seems to play a privileged role in the generation of affective responses to taste stimuli relative to the thalamocortical pathway, notwithstanding the caveat of the complex effect of VPMpc lesions on TR elicited by quinine. Whether GC is critical in maintaining performance in taste discrimination tasks remains to be explicitly tested.

4. Experimental Procedures

All procedures were performed in accordance with National Institutes of Health guidelines and with approval of the Institutional Animal Care and Use Committees at Stetson University and the Florida State University.

4.1. Animals

Sprague Dawley rats (N=20, Charles River Laboratories) weighing between 360–381 g at time of surgery were housed individually in polycarbonate cages on 12:12-h light-dark cycle. Standard lab chow (Teklad Rodent Diet, Harlan Laboratories) and distilled water were available ad libitum. Following the surgical session, food pellets moistened with dH_20 were provided along with the standard dry chow.

4.2. Surgical procedures

4.2.1. Lesion production—Surgeries were performed under ketamine hydrochloride (125.0 mg/kg) and xylazine (5.0 mg/kg) anesthesia (i.m.) with supplemental doses given as needed. The rat's head was placed in a non-traumatic stereotaxic device and the skull was exposed, cleaned and leveled using bregma and lambda as landmarks. Using bregma as the point of origin, we drilled access holes for glass micropipette placements. The dura, observed through the access holes, was punctured with a needle for ease of penetration. The glass micropipette (diameter ~0.40 μm) was attached to a 1.0 μl Hamilton syringe (Model 7001) and filled with either ibotenic acid (IBO, 20 mg/ml in phosphate buffered saline, PBS) or just PBS (for SHAM surgeries). Two injections were made on each side of the brain. The coordinates for the first injection site were: AP, +1.5 mm; ML, ± 5.2 mm; and DV, −6.4 mm. The coordinates for the second injection site were: AP, +0.5 mm; ML, ± 5.7 mm; and DV, −6.6 mm. At each site, 0.18 μl ibotenic acid or PBS was administered in three equi-volume infusions each separated by ~2 min. One rat died just prior to the neurotoxic lesion surgery.

4.2.2. Intraoral cannula surgeries—Concurrent with the ibotenic acid lesion (or PBS) surgery, two intraoral cannulae were implanted bilaterally (Grill and Norgren, 1978a, King et al., 1999). To maintain patency and prevent infection, the cannulae were cleaned daily. Immediately following and for three days after the surgical session animals were given

meloxicam (0.3 mg/kg, SC) and penicillin (30,000 units, SC). The postoperative recovery duration was at least two weeks.

4.3. TR procedures

4.3.1. TR testing—Animals were individually placed in the behavioral arena, an opaque cylinder (diameter, 280 mm) atop a transparent Plexiglas floor with a mirror angled underneath to allow for viewing and videotaping of the TR responses of the animal. For 3 days, the rats were habituated to the arena and the intraoral administration of fluid into the oral cavity. On these habituation days, before the rat was placed in the behavioral chamber, one of its two cannulae was connected via PE tubing to a swivel centered in the lid of the behavioral chamber. Another piece of PE tubing was used to connect the swivel to a syringe on an infusion pump (Harvard Apparatus). After a 5-min habituation period, the pump was turned on and the rat received an infusion of dH_2O at a rate of 0.23 ml/min over a 60-s period. After another 5 min, a second 60 sec infusion of dH_2O was given. The animal was then returned to its home cage. On the final day of habituation, all oromotor and somatic behaviors responses (see *4.3.2*) were videotaped (Panasonic AW-E300 camera and SLV-R10000 video cassette recorder) during the second dH_2O infusion for subsequent TR analysis.

Two successive test days occurred immediately after the final habituation day. These sessions emulated the procedure used on the final habituation day except that during the second infusion a test stimulus was given (1.0 M sucrose on the first test day and 0.003 M quinine hydrochloride on the second test day). After several weeks, the same 3-day habituation procedure was conducted followed by 2 more test days on which lower concentrations of the test solutions were delivered (0.1 M sucrose (first test day) and 0.0003 M quinine (second test day)).

4.3.2. TR scoring—The scoring of TR was conducted by an experimenter naïve to the surgical condition of the animal and to the taste stimulus being delivered. For each animal, a total of six 60-s video segments from the second infusion periods on the 2 final habituation days (dH_20) and the 4 test days (0.1M and 0.01M sucrose, and 0.003M and 0.0003M quinine) were viewed in slow motion to count the TR behaviors. Aversive and ingestive responses were scored using established behavioral classifications (Spector et al., 1988). Aversive behaviors included gapes, chin rubs, forelimb flails, and head shakes. Ingestive behaviors included mouth movements, tongue protrusions, lateral tongue protrusions, and paw licks. When possible, paw licks were counted, but at times the paws obscured a clear view of the tongue. When this occurred, paw lick duration was measured and because paw licks occur at about a rate of ~6 licks per second (Grill and Norgren, 1978a; Spector et al., 1988), paw lick duration (in s) was multiplied by 6. These corrected scores were added to the number of discrete paw licks observed for a more accurate indication of total paw licking. Total Aversive Scores and Total Ingestive Scores were calculated by summing the occurrences of the individual behaviors listed above.

4.4. Histological procedures

Within 2 days following the final test day of the second week of testing, animals were anesthetized with an overdose of Somnasol (5.0 mg/kg) and then perfused transcardially with PBS followed by 4% paraformaldehyde and 0.8% methyl alcohol in PBS. Brains were removed, post-fixed for at least 24 h in the same fixative, and transferred to formalin containing 10% glycerol for at least 72 h for cryoprotection. A freezing microtome was used to cut serial coronal sections (50 μm thick). The free-floating sections were post-fixed for 30 min, and then rinsed and mounted on gelatin-coated slides. The sections were left to air-dry for several days and were then Nissl stained (Thionin) and coverslipped.

4.5. Brain lesion analysis

4.5.1 Delimitation of GC and areas surrounding GC—The GC was defined as the regions of dysgranular (DI) and agranular (AI) insular cortex dorsal to the rhinal fissure and between +0.2 and +2.3 mm anterior to bregma and bordered medially by the claustrum and the external capsule, and laterally by the cortical surface of the brain. This region was delimited because throughout its extent taste-responsive neurons have been identified and gustatory thalamic terminations have been observed (Cechetto and Saper, 1987; Hanamori et al., 1998; Kosar et al., 1986a, 1986b; Yamamoto et al, 1980, 1985, 1989). The center, or "core" of GC, was defined as the regions of DI and AI positioned between +0.6 to +1.8 mm from bregma. In this core region, taste-responsive neurons are highly concentrated and have been identified in freely behaving rats (e.g., Accolla, et al, 2007; Fontanini and Katz, 2006; Katz et al., 2001; MacDonald et al., 2012; Maier and Katz, 2013; Stapleton et al., 2006). Only those GCX rats that had at least 50% damage to GC and at least 70% damage to the core region were included in the behavioral analysis.

4.5.2. Quantification of lesions using a high-spatial resolution lesion mapping program

4.5.2.1 Lesion Mapping: To map the size and location of the lesion in each hemisphere a custom lesion mapping system (Schier et al., 2014), which divided the GC and surrounding tissue into grid cells was employed to produce a high-spatial resolution 2-D reconstruction of each lesion. A viewer who was unaware of the surgical condition of the animals or the behavioral outcomes constructed the 2-D grid maps.

The approximate anterior/posterior (AP) location of each 50 μm coronal brain section containing the lesion and/or GC section relative to bregma was determined by first measuring the distance between two standard sections: 1) the section in which the corpus callosum first joins at midline; and 2) the section just rostral to the joining of the anterior commissure across the midline. That distance was divided by 2.3 mm (the actual distance based on Paxinos and Watson (2007)) and each section's position was adjusted by that factor. Then, each section containing either GC and/or lesion was carefully examined using a Leica microscope (model DMRB, McBain Instruments) equipped with Neurolucida software (version 9, MBF BioScience) and was mapped onto the grid to the nearest 50 μm on the AP axis.

An example of how a single coronal section was subdivided and mapped onto the 2D grid is shown in Fig. 7 while Fig. 2 provides examples of the lesion maps from each hemisphere of a representative GCX rat. Each row on the maps represents a 50 μm section of brain tissue and each large column represents one of the subregions of GC (DI and AI, above the rhinal fissure) or the surrounding tissue in the dorsal-ventral plane (granular insular cortex (GI), dorsal (D), ventral (V)). D and V subregions represent the areas extending the approximate height of GC in each respective direction. Each of the subregions (GI, DI, AI, D and V) was further divided into medial-lateral thirds. The smaller subcolumns on the maps represent these three medial-lateral partitions and, when appropriate, the grid cells for the claustrum (C) and an area medial to it (M, that extends the approximate mediolateral width of GC).

4.5.2.2. Lesion Scoring: With the grid drawn, the neuronal tissue in GC and the surrounding areas was assessed for sufficient damage. The evaluation of neural damage was also conducted by an experimenter naïve to the surgical condition of the animals or the behavioral results. The lesion contained within each grid cell was assigned a lesion score of '1.0' if all the neural tissue within that grid cell was damaged (indicated by red in Figs. 2 and 7), a lesion score of '0.5' if at least half of the neural tissue was damaged (indicated by yellow in Figs. 2 and 7) and a lesion score of '0' if less than half the neural tissue in the grid cell was damaged (indicated by white in Figs. 2 and 7). This lesion scoring procedure was repeated across each 50 μm section containing GC and/or lesion. Each hemisphere was mapped separately.

A lesion ratio score was used to determine the size of the GC lesion in each hemisphere. Lesion ratio scores were calculated by first totaling the lesion scores assigned to each grid cell within GC and then dividing this value by the total number of possible cells comprising GC. Likewise, a lesion ratio score was used to determine the size of the lesion within the "core" of the GC. A lesion was deemed successful if > 50% of GC and > 70% of the GC "core" were damaged in both hemispheres.

4.5.2.3 Lesion symmetry: To assess the symmetry of the bilateral lesions for a given rat, the lesion scores (0, 0.5, and 1.0) from the left and right hemispheres were compared on a grid-cell by grid-cell basis. The lower of the two scores for corresponding grid cells throughout the extent of GC and/or lesion was used to generate a third map, a 2D representation of the left-right hemispheric symmetry of the lesion for a given rat (Fig. 3).

To visualize the similarity of the bilateral lesions across animals, the hemispheric symmetry maps computed for the 10 GCX rats were combined to form an aggregate overlap map in which the average lesion score for each grid cell of the symmetry maps was calculated (Fig. 4).

4.6. Statistical analyses

Statistical analyses were performed using SYSTAT 8.0 statistical programs (SPSS, Inc., Chicago, IL, USA). Total Ingestive Scores and Aversive Scores were analyzed with 2-way (lesion X stimulus concentration) repeated measure ANOVAs. Two sample t-tests were used to compare the amount of time elapsed before the first gape to quinine was expressed, and to compare Total Ingestive Scores in response to water stimulation during the first and second

testing weeks. Bonferroni correct paired t-tests were used to assess any differences in Total Ingestive Scores in response to water versus each of the two concentrations of sucrose for each surgical group. The statistical rejection criterion was $P < 0.05$.

Acknowledgments

This work was supported by funding from the National Institute on Deafness and Other Communication Disorders (R01-DC009821 (to A.C.S.). The authors would like to thank Dr. Michael S. King, Stetson University, for his help with the intraoral cannula surgeries.

References

Accolla R, Bathellier B, Petersen CC, Carleton A. Differential spatial representation of taste modalities in the rat gustatory cortex. J Neurosci. 2007; 27(6):1396–1404. [PubMed: 17287514]

Accolla R, Carleton A. Internal body state influences topographical plasticity of sensory representations in the rat gustatory cortex. Proc Natl Acad Sci. 2008; 105(10):4010–5.10.1073/pnas.0708927105 [PubMed: 18305172]

Allen GV, Saper CB, Hurley KM, Cechetto DF. Organization of visceral and limbic connections in the insular cortex of the rat. J Comp Neurol. 1991; 311(1):1–16. [PubMed: 1719041]

Benjamin RM. Cortical taste mechanisms studied by two different test procedures. J Comp Physiol Psychol. 1955a; 48:119–122. [PubMed: 14367584]

Benjamin RM. The effect of fluid deprivation on taste deficits following cortical lesions. J Comp Physio Psych. 1955b; 48:502–505.

Benjamin RM, Akert K. Cortical and thalamic areas involved in taste discrimination in the albino rat. J Comp Neurol. 1958; 111:231–260. [PubMed: 13798908]

Benjamin RM, Pfaffmann C. Cortical localization of taste in albino rat. J Neurophysiol. 1955; 18:56–64. [PubMed: 13222157]

Berridge KC. Food reward: Brain substrates of wanting and liking. Neurosci Biobehav Rev. 1996; 20:1–25. [PubMed: 8622814]

Berridge KC. Measuring hedonic impact in animals and infants microstructure of affective taste reactivity patterns. Neurosci Biol Behav Rev. 2000; 24:173–198.

Braun JJ, Lasiter PS, Kiefer SW. The gustatory neocortex of the rat. Physiol Psychol. 1982; 10:13–45.

Cechetto DF, Saper CB. Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. J Comp Neurol. 1987; 262:27–34. [PubMed: 2442207]

Chen X, Gabitto M, Peng Y, Ryba NJ, Zuker CS. A gustotopic map of taste qualities in the mammalian brain. Science. 2011; 333(6047):1262–1266.10.1126/science.1204076 [PubMed: 21885776]

Craig W. Appetites and aversions as constituents of instincts. Biol Bull. 1918; 34:91–107.

Cromwell HC, Berridge KC. Where does damage lead to enhanced food aversion: the ventral pallidum/substantia innominata or lateral hypothalamus? Brain Res. 1993; 624(1–2):1–10. [PubMed: 8252379]

Davis JD. The effectiveness of some sugars in stimulating licking behavior in the rat. Physiol Behav. 1973; 11(1):39–45. [PubMed: 4732426]

de Araujo IE, Simon SA. The gustatory cortex and multisensory integration. Int J Obes (Lond). 2009; 33(Suppl 2):S34–43.10.1038/ijo.2009.70 [PubMed: 19528978]

Dunn LT, Everitt BJ. Double dissociations of the effects of amydala 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]

Fontanini A, Katz DB. State-dependent modulation of time-varying gustatory responses. J Neurophysiol. 2006; 96(6):3183–3193. [PubMed: 16928791]

Flynn FW, Grill HJ, Schwartz GJ, Norgren R. Central gustatory lesions: I. Preference and taste reactivity tests. Behav Neurosci. 1991; 105(6):933–943. [PubMed: 1663764]

Grill, HJ.; Berridge, KC. Taste reactivity as a measure of the neural control of palatability. In: Sprague, JM.; Epstein, AN., editors. Progress in Psychobiology and Physiological Psychology. Academic Press; Orlando: 1985. p. 1-61.

Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res. 1978a; 143:263–279. [PubMed: 630409]

Grill HJ, Norgren R. The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and decerebrate rats. Brain Res. 1978b; 143:281–297. [PubMed: 630410]

Grill, HJ.; Spector, AC.; Schwartz, GJ.; Kaplan, JM.; Flynn, FW. Evaluating taste effects on ingestive behavior. In: Toates, F.; Rowland, N., editors. Techniques in the Behavioral and Neural Sciences, vol. 1: Feeding and Drinking. Elsevier; Amsterdam: 1987. p. 151-188.

Grossman SE, Fontanini A, Wieskopf JS, Katz DB. Learning-related plasticity of temporal coding in simultaneously recorded amygdala-cortical ensembles. J Neurosci. 2008; 28(11):2864–2873.10.1523/JNEUROSCI.4063-07 [PubMed: 18337417]

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]

Hashimoto K, Spector AC. Extensive lesions in the gustatory cortex in the rat do not disrupt the retention of a presurgically conditioned taste aversion and do not impair unconditioned concentration-dependent licking of sucrose and quinine. Chem Senses. 2014; 39(1):57–71.10.1093/chemse/bjt054 [PubMed: 24226296]

Katz DB, Simon SA, Nicolelis MA. Dynamic and multimodal responses of gustatory cortical neurons in awake rats. J Neurosci. 2001; 21(12):4478–4489. [PubMed: 11404435]

Katz DB, Simon SA, Nicolelis MA. Taste-specific neuronal ensembles in the gustatory cortex of awake rats. J Neurosci. 2002; 22(5):1850–1857. [PubMed: 11880514]

Kida I, Iguchi Y, Hoshi Y. Blood oxygenation level-dependent functional magnetic resonance imaging of bilateral but asymmetrical responses to gustatory stimulation in the rat insular cortex. Neuroimage. 2011; 56(3):1520–1525.10.1016/j.neuroimage.2011.03.007 [PubMed: 21396461]

King CT, Garcea M, Spector AC. Restoration of quinine-stimulated Fos-immunoreactive neurons in the central nucleus of the amygdala and gustatory cortex following reinnervation or cross-reinnervation of the lingual taste nerves in rats. J Comp Neurol. 2014; 522(11):2498–2517.10.1002/cne.23546 [PubMed: 24477770]

King CT, Travers SP, Rowland NE, Garcea M, Spector AC. Glossopharyngeal nerve transection eliminates quinine-stimulated fos-like immunoreactivity in the nucleus of the solitary tract: implications for a functional topography of gustatory nerve input in rats. J Neurosci. 1999; 19(8):3107–21. [PubMed: 10191326]

Kosar E, Grill HJ, Norgren R. Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. Brain Res. 1986a; 379:329–341. [PubMed: 3742225]

Kosar E, Grill HJ, Norgren R. Gustatory cortex in the rat. II. Thalamocortical projections. Brain Res. 1986b; 379:342–352. [PubMed: 3742226]

MacDonald CJ1, Meck WH, Simon SA. Distinct neural ensembles in the rat gustatory cortex encode salt and water tastes. J Physiol. 2012; 590(Pt 13):3169–84.10.1113/jphysiol.2012.233486 [PubMed: 22570382]

Maier JX, Katz DB. Neural dynamics in response to binary taste mixtures. J Neurophysiol. 2013; 109(8):2108–17.10.1152/jn.00917.2012 [PubMed: 23365178]

Norgren R, Wolf G. Projections of thalamic gustatory and lingual areas in the rat. Brain Res. 1975; 92(1):123–9. [PubMed: 1174938]

Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. 6. Academic Press; New York: 2007.

Peciña S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci. 2005; 25(50):11777–11786. [PubMed: 16354936]

Peciña S, Smith KS, Berridge KC. Hedonic hot spots in the brain. Neuroscientist. 2006; 12(6):500–511. [PubMed: 17079516]

Pfaffmann C, Norgren R, Grill HJ. Sensory affect and motivation. Ann NY Acad Sci. 1977; 290:18–34. [PubMed: 276291]

Sadacca BF, Rothwax JT, Katz DB. Sodium concentration coding gives way to evaluative coding in cortex and amygdala. J Neurosci. 2012; 32(29):9999–10011.10.1523/JNEUROSCI.6059-11.2012 [PubMed: 22815514]

Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. J Comp Neurol. 1982; 210(2):163–173. [PubMed: 7130477]

Schier LA, Hashimoto K, Bales MB, Blonde GD, Spector AC. High-resolution lesion-mapping strategy links a hot spot in rat insular cortex with impaired expression of taste aversion learning. Proc Natl Acad Sci. 2014; 111(3):1162–1167.10.1073/pnas.1315624111 [PubMed: 24395785]

Smith JC. The history of the “Davis Rig”. Appetite. 2001; 36:93–98. [PubMed: 11161349]

Smith KS, Berridge KC. The ventral pallidum and hedonic reward: Neurochemical maps of sucrose “liking” and food intake. J Neurosci. 2005; 25(38):8637–8649. [PubMed: 16177031]

Smith KS, Berridge KC. Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. J Neurosci. 2007; 27(7):1594–1605. [PubMed: 17301168]

Spector AC. Linking gustatory neurobiology to behavior in vertebrates. Neurosci Biobehav Rev. 2000; 24(4):391–416. [PubMed: 10817842]

Spector, AC. Psychophysical evaluation of taste function in non-human mammals. In: Doty, RL., editor. Handbook of Olfaction and Gustation. 2. Marcel Dekker; New York: 2003. p. 861-879.

Spector AC, Breslin P, Grill HJ. Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste-visceral associations. Behav Neurosci. 1988; 102(6):942–952. [PubMed: 2850815]

Stapleton JR, Lavine ML, Wolpert RL, Nicolelis MA, Simon SA. Rapid taste responses in the gustatory cortex during licking. J Neurosci. 2006; 26(15):4126–38. [PubMed: 16611830]

Touzani K, Taghzouti K, Velley L. Increase of the aversive value of taste stimuli following ibotenic acid lesion of the central amygdaloid nucleus in the rat. Behav Brain Res. 1997; 88(2):133–42. [PubMed: 9404622]

Travers JB, Norgren R. Electromyographic analysis of the ingestion and rejection of sapid stimuli in the rat. Behav Neurosci. 1986; 100(4):544–555. [PubMed: 3741605]

Yamamoto T, Matsuo R, Kawamura Y. Localization of cortical gustatory area in rats and its role in taste discrimination. J Neurophysiol. 1980; 44(3):440–455. [PubMed: 7441309]

Yamamoto T, Yuyama N, Kato T, Kawamura Y. Gustatory responses of cortical neurons in rats. II. Information processing of taste quality. J Neurophysiol. 1985; 53(6):1356–1369. [PubMed: 4009223]

Yamamoto T, Matsuo R, Kiyomitsu Y, Kitamura R. Taste responses of cortical neurons in freely ingesting rats. J Neurophysiol. 1989; 61:1244–1258. [PubMed: 2746324]

Highlights

- Insular cortex lesions in rats were analyzed with a detailed mapping system
- Extensive gustatory cortex lesions did not impair quinine-stimulated taste reactivity
- Extensive gustatory cortex lesions did not impair sucrose-stimulated taste reactivity
- The gustatory cortex is not necessary for normal affective evaluation of taste stimuli

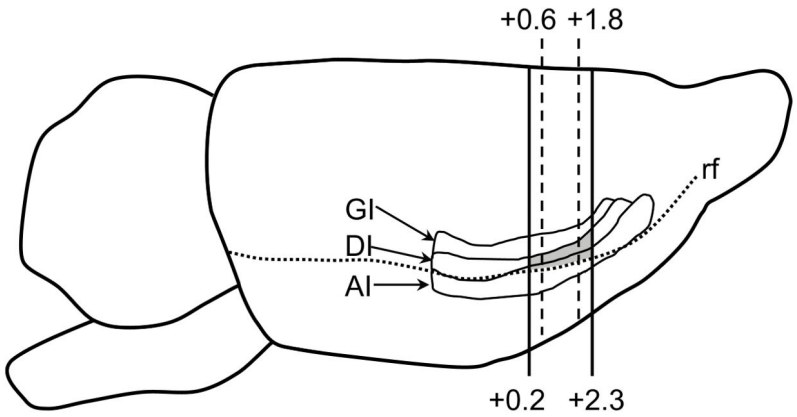

Fig. 1. Localization of the gustatory cortex (GC) within the rat insular cortex

The approximate location of the conventionally defined GC (gray region bounded by the solid lines) and the central area of GC that was operationally referred to as the "core" (dotted lines) within the rat insular cortex as shown in a lateral view. Numbers represent distance in mm from bregma along the anterior-posterior axis based on Paxinos and Watson (2007). AI, agranular insular cortex; DI, dysgranular insular cortex; GI, granular insular cortex; rf, rhinal fissure.

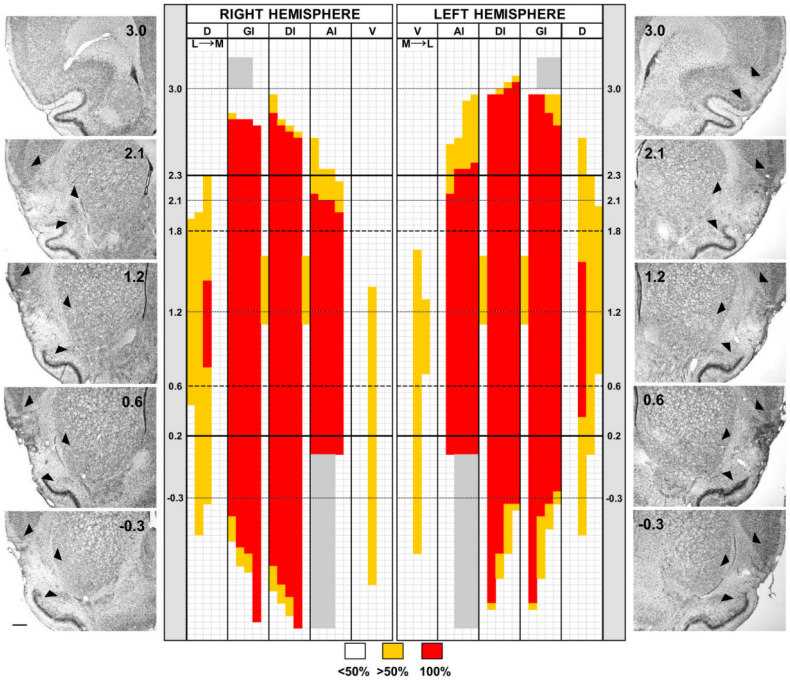

Fig. 2. Representative bilateral lesion and high-resolution 2-D lesion maps

Photomicrographs taken at specific A/P coordinates relative to bregma throughout the left and right hemispheres of a GCX rat illustrate the extent of the lesions produced by bilateral ibotenic acid injections targeting the GC. The black arrowheads note the approximate borders of the lesion. High-resolution 2-D lesion maps detailing the extent of the damage throughout the GC and surrounding areas in the right and left hemispheres are also shown. The solid black horizontal lines indicate the levels of the posterior (+0.2 mm, shown in photomicrograph) and anterior (+2.3 mm) borders of GC, and the dashed black horizontal lines (at +0.6 mm and +1.8 mm) mark the boundaries of the center or “core” of GC. The smaller dotted lines are additional reference points and indicate the levels at which the other photomicrographs were taken. Gray grid cells represent areas where the associated cortical regions do not exist and therefore no lesion score can be recorded, as per the stereotaxic atlas. Color scale below the maps denotes the percent of tissue damage in each grid cell. Refer to *4.5.2.2* for more detail. Abbreviations: AI, agranular insular cortex; D, dorsal to the insular cortex, DI, dysgranular insular cortex; GI, granular insular cortex; L, lateral; M, Medial; V, ventral to the rhinal fissure. Scale bar = 500 μm.

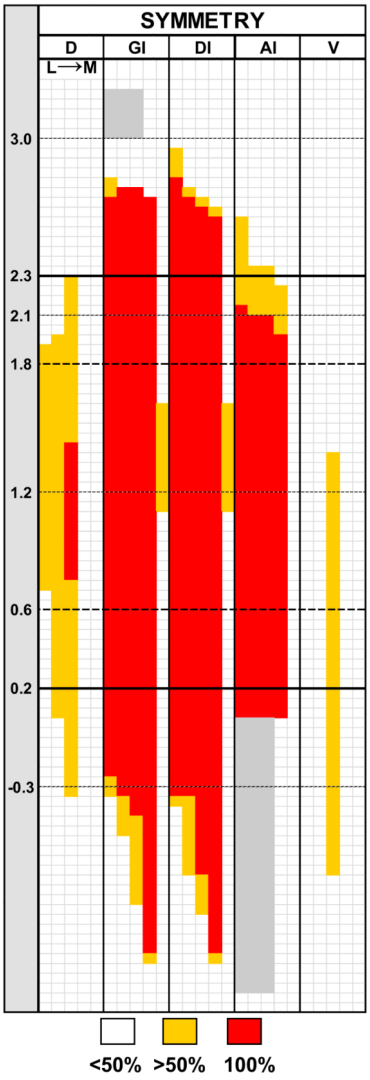

Fig. 3. Symmetry map

A representative symmetry map generated from the left and right hemisphere lesion maps depicted in Fig. 2. Roughly 98% of the GC and 100% of the GC core was destroyed bilaterally in this animal. Refer to *4.5.2.3* for a description of the procedure used to create the symmetry maps. Color scale below the map denotes the percent of tissue damage in each grid cell. Refer to *4.5.2.2* for more detail.

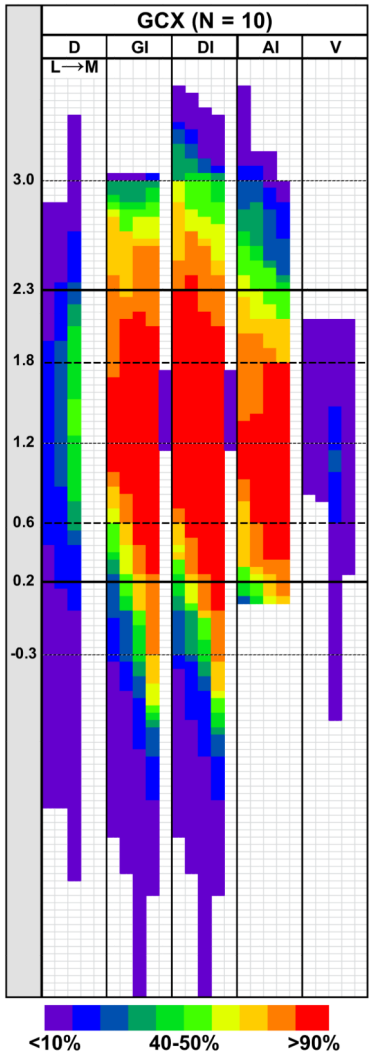

Fig. 4. Overlap map

Computed from the symmetry maps for the 10 animals whose lesions were deemed successful (≥ 50% of GC and ≥70% of the GC "core" damaged in both hemispheres), this overlap map shows the average size and location of these lesions, which were very large, encompassing ~94% of the GC and ~98% of its core, on average. The colors in the scale below the map signify the average percent of tissue damage sustained within each grid cell across the 10 animals.

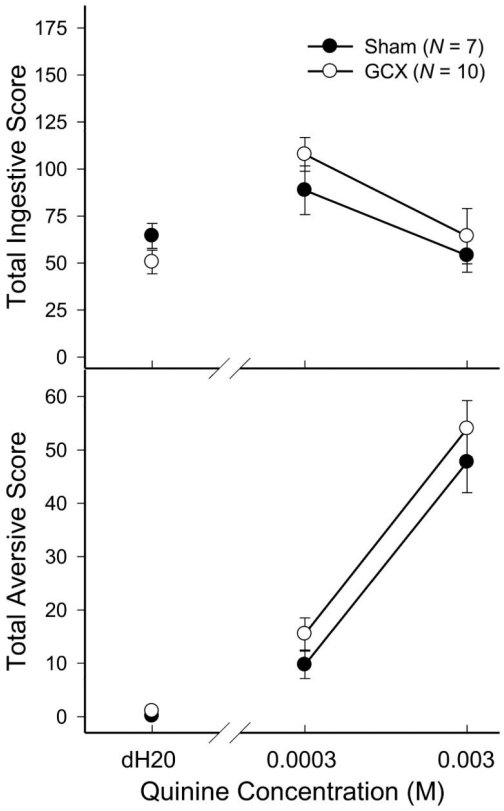

Fig. 5. Mean (± SE) total number of TR behaviors elicited during 60 sec intraoral infusions of different concentrations of quinine

The extensive GC lesions did not disrupt either (a) ingestive or (b) aversive TR responses to this bitter substance.

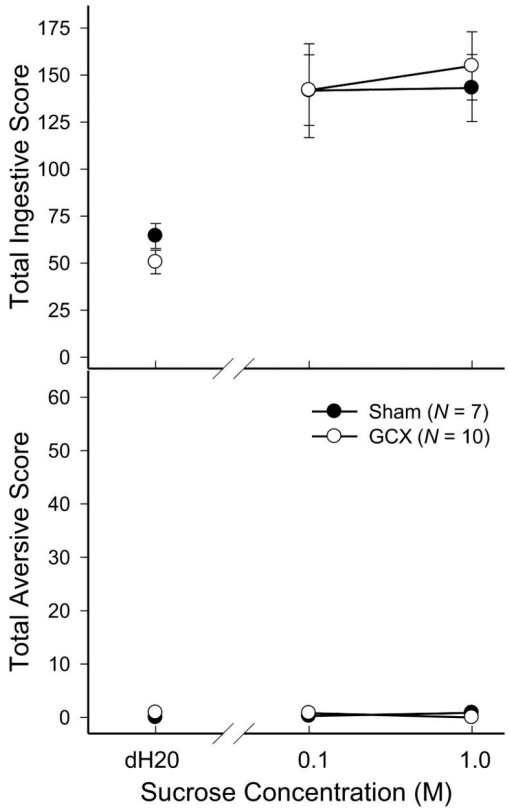

Fig. 6. Mean (± SE) total number of TR behaviors elicited during a 60-s infusion of different concentrations of sucrose

No differences in either (a) ingestive or (b) aversive TR responses to sucrose were observed between surgical groups.

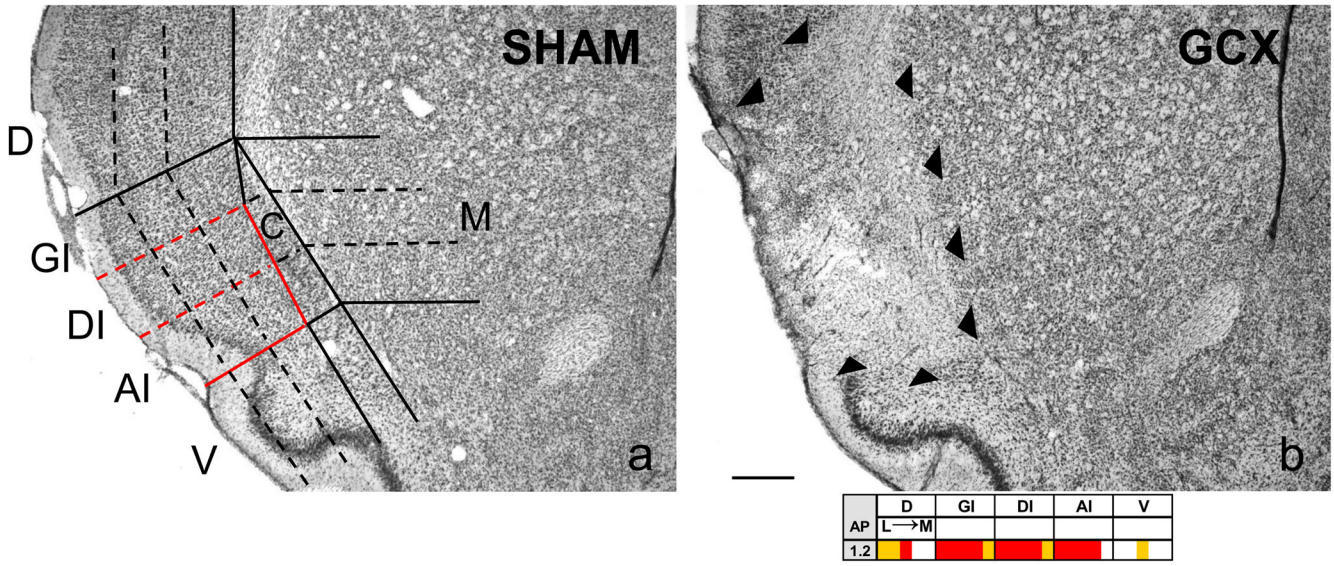

Fig. 7. Custom grid system used for lesion analysis

(a) Photomicrograph of a representative SHAM coronal section (+1.2 mm from bregma) showing the custom grid overlay. The GC (DI and AI) is outlined in a solid red line. (b) Photomicrograph of a GCX coronal section (+1.2 mm from bregma) showing neural damage that met the criteria for a successful lesion. Black arrowheads roughly demarcate the lesion. The corresponding custom grid map (in color) is also illustrated. The row shown represents the 50 μm tissue section in the photomicrograph. The large, bolded columns each represent one of the subdivisions of GC (DI, AI) or the surrounding tissue (GI, D, V). The smaller sub-columns represent the individual grid cells within each of these subdivisions and, when appropriate, the grid cells for the C and the M subdivision. Red denotes complete neural damage within that grid cell; yellow indicates that at least half of the neural tissue was damaged; and white indicates that less than half the neural tissue in the grid cell was damaged. Abbreviations: AI, agranular insular cortex (dorsal to the rhinal fissure); C, claustrum (outlined column just medial to AI, GI, and DI); D, dorsal to insular cortex; DI, dysgranular insular cortex; GI, granular insular cortex; M, medial to claustrum; V, ventral to rhinal fissure; based on Paxinos and Watson (2007). Scale bar = 500 μm.

Table 1

Mean (± SE) numbers of ingestive TR responses to dH_20, sucrose, and quinine by lesion status.

	TOTAL INGESTIVE SCORE	MM	TP	LTP	PLS
dH_20[a]					
SHAM (*N* = 7)	64.43(6.65)	35.14(5.30)	22.29(4.46)	4.07(0.90)	2.93(2.93)
GCX (*N* =10)	50.55(6.26)	29.20(3.89)	13.30(2.94)	4.35(1.53)	3.70(3.48)
0.1M Sucrose					
SHAM	141.71(24.94)	57.00(14.90)	61.43 (9.79)	16.71(4.37)	6.57(3.08)
GCX	139.60(18.75)	57.90(9.72)	59.30(15.95)	14.80(1.57)	7.60(3.76)
1.0 M Sucrose					
SHAM	143.14(17.81)	58.00(13.43)	54.57(6.93)	22.57(2.56)	8.00(4.91)
GCX	147.30(17.54)	65.30(7.44)	60.20(13.23)	17.80(3.78)	4.00(2.62)
0.0003M Quinine					
SHAM	88.71(12.94)	46.86(10.31)	27.86(5.69)	12.00(3.63)	2.00(1.36)
GCX	107.80(8.95)	65.10(8.35)	31.10(4.13)	9.90(1.73)	1.70(1.70)
0.003 M Quinine					
SHAM	54.00(8.93)	28.57(6.72)	18.86(3.88)	6.43(1.56)	0.14(0.14)
GCX	64.30(14.72)	38.80(7.48)	21.80(6.55)	3.70(2.23)	0.00

[a]Note: dH_20 values reflect the average number of responses on the final day of habituation during the first testing week and during the second testing week.

Abbreviations: MM, mouth movements; TP, tongue protrusions; LTP, lateral tongue protrusions; PL, paw licks

Table 2

Mean (± SE) numbers of aversive TR responses to dH_20, sucrose and quinine by lesion status.

	TOTAL AVERSIVE SCORE	G	CR	FF	HS
dH_20[a]					
SHAM (*N* = 7)	0.14(0.09)	0.00	0.00	0.14(0.09)	0.00
GCX (*N* = 10)	1.05(0.67)	0.55(0.40)	0.00	0.25(0.13)	0.25(0.17)
0.1M Sucrose					
SHAM	0.29(0.18)	0.00	0.00	0.29(0.18)	0.00
GCX	0.80(0.47)	0.50(0.40)	0.00	0.20(0.20)	0.10(0.10)
1.0 M Sucrose					
SHAM	0.86 (0.70)	0.86(0.70)	0.00	0.00	0.00
GCX	0.00	0.00	0.00	0.00	0.00
0.0003M Quinine					
SHAM	9.71(2.58)	9.43(2.64)	0.00	0.29(0.18)	0.00
GCX	15.50(3.02)	14.00(2.72)	0.40(0.22)	0.60(0.40)	0.50(0.31)
0.003 M Quinine					
SHAM	47.71(5.72)	32.29(5.55)	13.29(0.97)	1.71(1.08)	0.43(0.30)
GCX	54.10(5.24)	41.10(3.80)	11.80(2.24)	0.80(0.61)	0.40(0.31)

[a]Note: dH20 values reflect the average number of responses on the final day of habituation during the first testing week and during the second testing week.

Abbreviations: G, gapes; CR, chin rubs; FF, forelimb flails; HS, head shakes