Published in final edited form as:

J Comp Neurol. 2020 November 01; 528(16): 2729–2747. doi:10.1002/cne.24928.

Chemospecific Deficits in Taste Sensitivity Following Bilateral or Right Hemispheric Gustatory Cortex Lesions in Rats.

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Abstract

Our prior studies showed bilateral gustatory cortex (GC) lesions significantly impair taste sensitivity to salts (NaCl and KCl) and quinine (“bitter”) but not to sucrose (“sweet”). The range of qualitative tastants tested here has been extended in a theoretically relevant way to include the maltodextrin, Maltrin, a preferred stimulus by rats thought to represent a unique taste quality, and the “sour” stimulus citric acid; NaCl was also included as a positive control. Male rats (Sprague-Dawley) with histologically confirmed neurotoxin-induced bilateral (BGCX, $n=13$), or left (LGCX, $n=13$) or right (RGCX, $n=9$) unilateral GC lesions and sham-operated controls (SHAM, $n=16$) were trained to discriminate a tastant from water in an operant two-response detection task. A mapping system was used to determine placement, size, and symmetry (when bilateral) of the lesion. BGCX significantly impaired taste sensitivity to NaCl, as expected, but not to Maltrin or citric acid, emulating our prior results with sucrose. However, in the case of citric acid, there was some disruption in performance at higher concentrations. Interestingly, RGCX, but not LGCX, also significantly impaired taste sensitivity, but only to NaCl, suggesting some degree of lateralized function. Taken together with our prior findings, extensive bilateral lesions in GC do not disrupt basic taste signal detection to all taste stimuli uniformly. Moreover, GC lesions do not preclude the ability of rats to learn and perform the task, clearly demonstrating that, in its absence, other brain regions are able to maintain sensory-discriminative taste processing, albeit with attenuated sensitivity for select stimuli.

Graphical Abstract

Using a taste signal detection task with a specialized gustometer, we found that extensive lesions bilaterally in the rat gustatory cortex (BGCX), or in the right GC (not shown), impair taste sensitivity (significant shift of EC_{50}) for NaCl but not for maltodextrin (Maltrin) or citric acid relative the sham-operated rats (SHAM). In addition to suggesting some degree of laterality of function in GC with respect to NaCl detection, these findings add to growing evidence suggesting that gustatory sites outside of GC can maintain normal taste sensitivity for select stimuli.

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Conflict of Interest: The authors declare no competing conflict of interest.

Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

Insular Cortex; Taste Detection; Taste Psychophysics; Gustatory System; RRID: RGD_737891; RRID: SCR_001775; RRID: SCR_010455

INTRODUCTION

One time-honored approach that has been applied to discern the functional significance of the gustatory cortex (GC) is the assessment of the behavioral consequences of selective damage to this higher order region of the central gustatory pathway in animal, especially rodent, models. Although there is evidence that bilateral GC lesions in rats disrupt the acquisition and expression of conditioned taste aversions (e.g. Yamamoto et al., 1980; Braun et al., 1981; Braun et al., 1982; Cubero et al., 1999; Stehberg and Simon, 2011; Schier et al., 2014), its effects on other taste-related behaviors are weak at best. For the most part, bilateral GC lesions in rats do not seem to have much of an effect on unconditioned taste preference and avoidance as assessed in intake tests (Braun et al., 1982; Dunn and Everitt, 1988; Benjamin and Pfaffmann, 1955; but see Benjamin and Ackert, 1959). Even in brief access taste tests, which minimize the contribution of postingestive events associated with intake measures, rats with lesions placed centrally in GC display normal concentration-dependent licking responses during very short duration trials of sucrose and quinine (Hashimoto and Spector, 2014). These results are consistent with the finding that rats with large lesions in GC display normal unconditioned oromotor and somatic responses to small volumes of sucrose and quinine infused directly into the oral cavity (King et al., 2015), a behavioral assay referred to as taste reactivity (see Grill and Norgren, 1978).

The failure of GC lesions in rats to disrupt normal unconditioned responding in brief-access licking assays and in taste reactivity tests suggest that this area of insular cortex is not necessary for unconditioned affective responsiveness to taste stimuli to be maintained, but this does not mean that they have no effect on taste perception per se. It is possible that the perceived intensity or quality of a taste stimulus is altered by disruption of cortical processing, despite the fact that taste-related motivation and affect remain unscathed. Investigators can assess sensory-discriminative function in animal models relatively independent of taste-driven affect by using the taste stimulus as a cue in a discrimination paradigm based on operant conditioning principles. Using a two-response operant task that requires immediate responses to very small volumes of stimuli, thus minimizing postingestive contributions, our lab previously showed that large, bilateral lesions in GC (~80% on average) impaired normal taste detection for NaCl and KCl. The same lesions retarded the acquisition of a NaCl vs. KCl discrimination, even though it was eventually learned (Blonde et al., 2015). Bales et al. (2015) replicated the impairment in KCl detection in rats with large bilateral lesions in GC (~91% damage) and these same rats also displayed a modest rightward shift in sensitivity to quinine, but, surprisingly, they displayed absolutely no impairment in the detection of sucrose.

The fact that sucrose taste sensitivity was unaffected by GC lesions which impacted other taste qualities raises the important question as to whether detectability of chemical stimuli representing other taste qualities would be affected by damage to this region of insular cortex. Accordingly, we further explored the role of GC in taste sensitivity in the rat, by extending the stimulus array tested to include citric acid, a representative “sour” stimulus that is normally avoided, and Maltrin, a maltodextrin stimulus highly preferred by rodents and thought to represent a unique taste quality (Nissenbaum and Sclafani, 1987; Sclafani, 2004). Importantly, because there is conflicting evidence for potential lateralization of cortical taste function (Small et al., 1997; Pritchard et al., 1999; Stevenson et al., 2013), we included groups that received unilateral GC lesions in either the right or left hemispheres.

MATERIALS AND METHODS

Subjects

Eighty-five (44 in phase one and 41 in phase two) male Sprague-Dawley rats (Charles River Laboratories, RRID: RGD_737891) weighing 290–356 g (323 ± 33 g) at the start of the experiment served as subjects. Due to small lesions in Phase 1, especially in the right hemisphere, a second phase was conducted to increase the number of animals with complete bilateral and right hemispheric lesions within GC. The rats were individually housed in polycarbonate cages in a room with a 12:12-h light:dark cycle that was climate-controlled. Every rat had access to a stainless-steel toy, which was not ingestible, as a form of enrichment. Ad libitum chow (Rodent Diet 5001; PMI) and deionized water (DW) were provided unless otherwise noted. All procedures were approved by the Animal Care and Use Committee at Florida State University and complied with the National Institutes of Health guidelines for humane handling of animals.

Stimuli

All taste stimuli were dissolved in DW and made fresh each day. All but MaltrinQD M580 Maltodextrin (Maltrin) were reagent-grade chemicals. Psychometric sensitivity functions were obtained for NaCl (Macron Fine Chemicals; 0.0002, 0.0004, 0.0008, 0.0015, 0.003, 0.006, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 M), Maltrin (Grain Processing Corporation; 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5, 10, and 20% w/v), and citric acid (Sigma Aldrich; 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5, 10, 20 mM).

Apparatus

We used computer-controlled devices known as gustometers (Spector et al., 2015) to test the taste sensitivity of rats to NaCl, Maltrin, and citric acid. The gustometer allows for the highly controlled delivery of fluid (~5 μ l) and measurement of immediate licking responses. The rat is contained in a chamber with the sides and back wall made from Plexiglas, a stainless-steel front panel with three equidistant slots, and a wire mesh floor. Through the center slot on the front panel, the rat has access to a borosilicate glass sample ball. As the ball rotates on a fixed axis, licks are registered via a force transducer that is attached to a mechanical arm driven by a motor. This mechanical arm allows the sample ball to be positioned in front of one of up to 14 polytetrafluoroethylene (PTFE) tubes (threaded through a turret), through which the sample stimuli are delivered onto the sample ball in precise volumes via an infusion pump. Stimulus delivery begins when the rat licks the sample ball twice within 250-ms upon which ~10 μ l is deposited to coat the sample ball. Thereafter, ~5 μ l of stimulus is delivered upon each subsequent lick. The mechanical arm moves the sample ball into a washing well where the ball is rinsed with DW and dried with pressurized air during the intertrial intervals.

Response balls are accessed through the slots on either side of the sample ball where DW reinforcement is delivered through tubing threaded through the ball and licks are registered with force transducers. A green cue light sits above each of the response balls and is visible via a circular cutout in the stainless-steel panel above the right and left slots. All three slots can be manually blocked with stainless-steel shutters.

The chamber sits inside a sound-attenuating box with a speaker that emits a masking noise to minimize auditory cues. A ventilation duct sits above the turret to minimize orthonasal olfactory cues from the sample stimuli. Of course, no amount of ventilation can prevent retronasal olfaction. A stainless-steel shield is attached to the front of the turret to block visual cues and a house light is anchored in the ceiling of the sound attenuation enclosure.

Surgery

Rats received bilateral (BGCX; Total, N=26; Phase 1, N=14; Phase 2, N=12), left unilateral (LGCX; Total, N=19; Phase 1, N=11; Phase 2, N=8), or right unilateral (RGCX; Total, N=23; Phase 1, N=11; Phase 2, N=12) infusions of ibotenic acid (20 mg/ml, dissolved in phosphate buffered saline [PBS]) or PBS alone (SHAM; Total, N=17; Phase 1, N=8; Phase 2, N=9) into gustatory cortex. One SHAM and one RGCX rat died during or shortly following surgery. An attempt was made to balance these groups as much as possible based on body weight and gustometer assignment.

All rats were initially anesthetized in an induction chamber with 5% isoflurane and then maintained in an anesthetized state via nosecone with 2–3% isoflurane while secured in a stereotaxic instrument with non-traumatic ear bars. A ~3 cm incision was made at the midline, exposing the skull, which was leveled by adjusting the bite bar to equalize the DV coordinates of bregma and lambda. As conventionally defined, GC stretches over 2 mm in the anterior/posterior (AP) dimension, so ibotenic acid was infused in two locations along the AP axis for one or both hemispheres depending on group assignment. The infusion was made with a Hamilton syringe that had a glass micropipette (~40–50 μm tip diameter) secured and sealed on its barrel via paraffin.

After determining bregma, small holes were drilled over the injection sites. Dura was carefully exposed and then a small cut was made using a 23G needle. The plunger was pressed to ensure the ibotenic acid or PBS was being delivered before lowering the pipette slowly into the brain. The rostral infusion (0.21 μl) was positioned at +1.5 mm AP, ± 5.2 mm ML, and -6.4 mm DV (Phase 1) or -6.8 mm DV (Phase 2) and the caudal infusion (0.18 μl) was positioned at +0.5 mm AP, ± 5.8 mm ML, and -6.6 mm DV (Phase 1) or -7.0 mm DV (Phase 2), relative to bregma. Each infusion took place over 10 minutes to facilitate even dispersion of the ibotenic acid. Each infusion began 2 min after the pipette was inserted. The pipette was removed 2 min after the infusion was complete. Wound clips were used to close the incision and were removed 7–10 days after surgery. On the day of and three days following surgery, to relieve pain and inflammation, the analgesic carprofen (5 mg/kg body mass, s.c.) was administered along with the antibiotic Gentamicin (8 mg/kg body mass, s.c.) to prevent infection.

Taste Detection

Psychometric taste sensitivity functions were derived for NaCl, Maltrin and citric acid (see Table 1). Animals were trained in a two-response operant taste discrimination task with a single concentration of the tastant and DW presented in a pseudorandom fashion in blocks of six trials, similar to Blonde et al. (2006), called “test sessions”. The probability of a taste stimulus presentation was 0.5. The test stimulus concentration was systematically lowered across test sessions (a modified method of limits procedure). Before training or testing on Monday, we removed water bottles from the home cage on Sunday afternoon and returned them on Friday after the session to provide ad libitum access to water over the weekend. Additional DW was delivered as described during the 30-min session in the gustometer each weekday. If rats were below 85% of their previous ad libitum body weight, or if they did not have a 30-min session in a gustometer, they were given access to DW for 1 h on the home cage.

Trial Structure.—The parameters for test sessions stayed constant across stimuli. The rat was required to lick the dry sample ball twice within 250 ms to initiate a trial and ensure active licking was occurring. The stimulus was then dispensed for up to 10 licks or for 3 s, whichever came first. After which, the rat had a limited amount of time (5 s) to respond to the left or right response balls to receive 20 licks of DW, which only occurred if the rat chose the correct side. If the rat chose the incorrect side or did not respond at all, it was punished with a timeout of 20 s and no DW reward was delivered. During an intertrial interval of ~8 s

the sample ball was rinsed and dried as described above. See Figure 1 for an illustration of the trial structure.

NaCl Detection.—To familiarize the animals with obtaining fluid from the sample and reinforcement balls, we included a lick training stage for the first three to four days of 30-min sessions. The rat had access to either the center (sample), left (response), or right (response) ball which delivered unlimited DW during the session. The other two balls were made inaccessible with shutters.

Next, the rat was trained to associate one response ball with the taste stimulus (0.4 M NaCl) and the other with DW. This was accomplished by presenting six sessions (three with NaCl alone and three with DW alone) during which the rat had access to the sample ball and the response ball that was assigned to that stimulus (left or right) while the other response ball was blocked by the shutter. After 10 licks of the sample stimulus, the response was reinforced with 20 licks of DW or 5 s, (whichever came first) from the response ball. The rat had 180 s to respond and if the rat did not lick the response ball within that time, the rat did not receive water reinforcement, but no timeout was given, and then a new trial began. The side that was assigned to the stimulus was counterbalanced across surgery groups.

After side training, the alternation stage began, during which the rat was presented with DW or 0.4 M NaCl from the sample ball and then chose one of the response balls, both of which were accessible. The same stimulus (either DW or NaCl) was repeatedly presented until the rat responded on the correct response ball for a set number of trials (nonconsecutive; criterion decreased over sessions from 8, 6, 4, to 2 correct trials) at which point the stimulus switched. During this phase the rat had 15 s to respond (referred to as a “limited hold”), and after an incorrect response or no response, there was a 20-s timeout.

During the next training stage, Random Training I, sample stimuli (DW or 0.4 M NaCl) were presented at random and the limited hold was reduced to 10 s. This stage lasted for two to three sessions and allowed for a more gradual change in the limited hold time. In Random Training II sample stimuli were presented at random but the limited hold time was reduced to 5 s. This last stage of training continued for one to thirteen sessions until all rats reached 80% correct overall on trials with a response.

Once training was completed, test sessions were conducted on Tuesdays and Thursdays, when all rats received DW and a single NaCl concentration that was successively lowered across days. Mondays, Wednesdays, and Fridays constituted control sessions when the rats received the lowest concentration of NaCl with which it performed 80%; this was done to maintain and monitor stimulus control of behavior throughout testing while taking individual differences in sensitivity and performance into account. Testing concentrations were lowered across sessions by approximately 0.27 to 0.32 $\log_{10}$ units starting at 0.4 M NaCl until all rats performed at or near chance (50%) which occurred by the time 0.00002 M NaCl was tested. At the end of testing for NaCl, if a rat had not performed 70% on the control day prior to a test day, that test day concentration was repeated and the data for both days were averaged.

Maltrin Detection.—After NaCl testing, rats were given up to six sessions (Random Training II) to familiarize them to the new stimulus (20% Maltrin) after which all rats performed 80% overall on trials with a response. Testing for Maltrin was very similar to testing for NaCl described above. Testing began with 20% Maltrin and then concentrations were subsequently lowered by approximately 0.30 log₁₀ units until all rats performed at or near chance which occurred by the time 0.04% Maltrin was tested.

Citric Acid Detection.—Following Maltrin testing, rats were given up to six sessions (Random Training II) with 10, 20, or 30 mM citric acid as the stimulus after which every rat performed 80% overall on all trials with a response. At first, 30mM was used but some animals reduced sample licks, so the citric acid concentration was lowered to promote more complete stimulus sampling. Testing for citric acid was nearly identical to NaCl and Maltrin. It began with 20 mM citric acid and was lowered by approximately 0.30 log₁₀ units until all rats performed at or near chance which happened when 0.02 mM citric acid was tested.

Water Test.—To ensure that rats were using only the chemical stimulus to guide responses, a “water test” was conducted after testing was complete for each stimulus for Phase 1 (three water tests in total) and after citric acid only for Phase 2. Although rats reached 50% (i.e. chance) performance during testing for NaCl and Maltrin for Phase 1, water tests were nonetheless conducted, though this may have been unnecessary because reaching 50% performance during testing is an indication that the rat is not using an extraneous cue to perform the task. Nevertheless, the water test did not appear to have an obvious detrimental effect on performance given that all rats performed well on the following control days. Because all rats reached chance levels for NaCl and Maltrin testing for Phase 2, water tests were deemed unnecessary after testing for those stimuli. The Phase 2 rats did receive a water test after citric acid testing to confirm that they were not using cues other than what was provided by the fluid stimulus. For the water test, each sample tube was filled with DW and arbitrarily assigned as the “tastant” or “non-tastant”. The test was otherwise the same as a regular test session. Theoretically, rats should perform at chance because there is a reliable chemical cue associated with the “tastant” sample.

NaCl Post-Detection Test.—To ensure that no learning or neural compensation occurred over time, two additional concentrations of NaCl were tested after citric acid detection testing for Phase 2. The concentrations that were repeated were 0.0125 M and 0.003 M NaCl based on the significant differences in performance found between surgical groups at those concentrations. All rats performed 80% overall in the days that flanked these test sessions when rats were given 0.4 M NaCl.

Histology

After testing in the gustometers was complete, each rat was anesthetized with an overdose of a euthanasia agent containing pentobarbital sodium (Somnasol). The rat was then transcardially perfused with 0.1 M saline followed by buffered formalin (4% paraformaldehyde and 0.8% methanol in PBS). Before the brain was removed from the skull, the head was placed in a stereotaxic instrument and leveled according to bregma and lambda. Then, a coronal cut was made perpendicular to the skull surface and just rostral to

lambda to provide a level and consistent plane for sectioning across animals. The brain was then removed from the skull and stored in the fixative for at least 72 h and was then sliced coronally on a vibratome at 50 μ m. Phase 1 brain sections were first rinsed with DW and then dipped in a 0.3% gelatin + 40% ethanol solution to aid in sticking to slides that were dipped twice in a 0.3% gelatin and 0.05% chromium potassium sulfate solution and dried overnight. However, the rinse and gel solution was not used for Phase 2 sections as it was deemed unnecessary. After all brain sections were mounted on double-subbed slides, they dried overnight and then were conventionally dehydrated and Nissl-stained using thionin and then coverslipped.

Lesion Analysis

The GC is conventionally defined as a region above the rhinal fissure, within agranular (AI) and dysgranular (DI) insular cortex that stretches from approximately +0.2 mm to 2.3 mm anteriorly from bregma and is just lateral to the claustrum and is where taste-responsive neurons and taste thalamic projections have been found (Kosar et al., 1986a; Kosar et al., 1986b; Cechetto and Saper, 1987; Hanamori et al., 1998). The center of GC, +1.8 to +0.6 relative to bregma, was defined as GC “core”, a region targeted in electrophysiological experiments on taste-responsive neurons in awake rats (Yamamoto et al., 1989; Katz et al., 2001; Fontanini and Katz, 2006; Stapleton et al., 2006; MacDonald et al., 2012; Maier and Katz, 2013). The lesions were analyzed using a Leica light microscope (model DMRB; McBain Instruments) along with Neurolucida software (MicroBrightField; <http://www.mbfbioscience.com/neurolucida>, RRID: SCR_001775) by an observer blind to the behavioral outcomes of a given rat.

A custom lesion mapping system was used to quantify the size, location, and bilateral symmetry (for the bilateral lesions only) of the lesions (see Figure 2). For a complete description of the system see Schier et al., 2014 and Schier et al., 2016. Using this system, we could divide the GC and adjacent regions into subdivisions based on the rat brain atlas (Paxinos and Watson, 2007) on a two-dimensional (2D) grid to precisely map the lesion damage. The grid had rows that represented 50 μ m brain sections (AP) and columns that represented a subdivision of insular cortex and surrounding areas. Aside from the GC (described above), areas that were represented included: granular insular cortex (GI), dorsal to GI (D), ventral to the rhinal fissure (V), medial to external capsule (M), and claustrum (C). The regions that border GC but do not have atlas-defined anatomical markers were assigned boundaries; for M, the ML border was equivalent to the width of GC on the same AP plane, and for D, the DV height of GC on the same AP plane was used to make the dorsal border. To increase the spatial resolution of the analysis, we divided GI, DI, AI, D, and V into five lateral (L)-to-medial (M) subfields represented in five subcolumns. Starting from the cortical surface and moving medially, the first 3 columns represent the L-to-M subfields of the IC, the fourth column represents the claustrum, and the fifth column represents all regions medial to the external capsule (which is just medial to the claustrum). All of the rows and columns combined fully represent all areas within and around GC on the 2D map. Each of these grid cells was given a score of 1, 0.5, or 0 (1 = complete lesion, 0.5 = at least half the tissue damaged, 0 = less than half or no lesion; also see Figures 2–4).

Our lesion mapping system was based on the stereotaxic coordinates and subdivisions associated with the Paxinos and Watson (2007) rat brain atlas. Our definition of gustatory cortex was based on the synthesis of data from the work of Cechetto and Saper (1987), Kosar et al. (1986a & 1986b), and Hanamori et al. (1998). Caution should be exercised when translating our AP and DV levels with other atlases such as that from Swanson (2018). Indeed, in the Swanson atlas, the brain region designated as GC is more expansive along the AP axis and the ventral border does not extend to the rhinal fissure. Nevertheless, the lesion maps presented here provide an objective depiction of the location of the lesions with respect to a specified set of stereotaxic coordinates and boundaries. One limitation is that these boundaries are based on Nissl-stained sections, whereas somewhat greater precision could be achieved by using immunohistochemistry to label neurons based on characteristic markers (see Van de Werd and Uylings, 2008).

Anatomical landmarks distinctly associated with one brain section, according to the rat brain atlas (Paxinos and Watson, 2007), were used to determine the best approximate AP coordinate (see Bales et al., 2015; Table 2). The AP distance between the landmarks as seen in the atlas was divided by the number of sections between those landmarks for each brain. The estimated thickness for each section was adjusted based on the number of sections between those landmarks to determine the AP coordinates for each section. Schier et al. (2014, 2016) further subdivided the gustatory cortex into anterior (IC₁, Figure 1) and posterior portions (IC₂, Figure 1). Because, in our study, the lesions targeted the entire gustatory cortex (IC₁+IC₂), we did not distinguish IC₂ and IC₃.

For the BGCX group, a symmetry map was created in which each corresponding grid cell between the left and right hemispheres was compared and the lesser score for each grid cell was conservatively assigned to a separate symmetry score map (see Figure 3 center panel). For example, if both grid cells had a score of 1, a score of 1 would be assigned on the symmetry map; if one hemisphere had a score of 0.5 and the other a score of 1 or 0.5, the symmetry map grid cell would be assigned 0.5; if one hemisphere had a score of 0 and the other a score of 1, 0.5, or 0, the symmetry map grid cell would be given a score of 0. The sum of each grid cell within a given region (e.g. GC) was used to determine lesion size in or outside the regions described above. The proportion of GC with lesion was determined by dividing the lesion score within GC with the total number of grid cells within GC. The proportion of the GC “core” with damage was calculated the same way.

Lesion overlap maps also allowed comparison of symmetry maps (average lesion size and location) across multiple rats and comparison across the lesions in the right or left hemispheres. First, because the AP coordinate for each brain section was calculated based on the number of sections between anatomical landmarks and therefore varied across brains, each row for each symmetry map was rounded to the nearest multiple of 10. The row was then divided into multiple 10 µm rows to standardize the AP coordinates across symmetry maps. For example, if one row was originally 50 µm of brain thickness it would then be expanded to 5 rows of 10 µm. Overlap maps were then created by averaging each corresponding grid cell of symmetry maps for a specific set of rats (e.g. all rats that reached lesion criteria). A color scale was used to represent the average lesion in each grid cell (See Figures 5, 6, and 7).

Experimental Design and Statistical Analysis

This experiment has a between-subjects design with male rats divided into a control (SHAM, $n=17$) or lesion group (BGCX, $n=26$; LGCX, $n=19$; RGCX, $n=23$). Of these, two rats died during or shortly after surgery (RGCX, $n=1$; SHAM, $n=1$), one died following NaCl detection testing (BGCX=1), and one died following CA testing (RGCX=1). As in our prior work, the only animals included in the behavioral analysis met the lesion criteria of 50% of GC and 70% of GC “core” destroyed. The final group sizes of rats which met the lesion criteria were: BGCX, $n=13$; LGCX, $n=13$; RGCX, $n=9$.

For NaCl, Maltrin, and citric acid, the proportion correct for stimulus and water trials with a response from that session for a single concentration were averaged across control and test sessions for each rat. Curves were fit to these data using a logistic equation representing performance as a function of concentration in SYSTAT (Version 13.2; <http://www.systat.com>, RRID: SCR_010455):

$$f(x) = \left[\frac{a - 0.5}{1 + 10^{(\log_{10} x - c)b}} \right] + 0.5$$

where x = stimulus concentration, a = asymptotic performance, b = slope, and $c = \log_{10}$ stimulus concentration at $\frac{1}{2}$ -asymptote (i.e. EC_{50}). The EC_{50} was operationally defined as the detection threshold because it represents the inflection point of the concentration-performance curve and therefore is best suited to measure experimentally induced lateral shifts representing increases or decreases in sensitivity. The asymptote parameter (a) represents a general performance estimate. It is useful to determine whether there is a decay in learning and or performance over and beyond any changes or lack thereof in the EC_{50} . A NaCl curve could not be fit to the data from one LGCX rat due to a very steep drop in performance between two concentrations. Accordingly, linear interpolation was used to place a performance data point directly in the middle of those concentrations to facilitate the regression. Likewise, a Maltrin curve could not be fit to the data for one RGCX rat due to a sharp drop in performance when the concentration was lowered from 10% to 5%. This was the case even after an interpolated performance data point was added between the two concentrations. Accordingly, we fit a line for the performance between those two concentrations and solved for the $\log_{10}$ stimulus concentration that represented $\frac{1}{2}$ of the performance observed at the 10% concentration, which was maximal for this animal (with 50% as the chance baseline). Therefore, there was no a or b parameters derived for that animal. All parameters were analyzed using t-tests (both uncorrected and Bonferroni corrected are presented) and average proportion correct was analyzed using two-way ANOVAs. One BGCX rat, that did not reach chance performance on the lowest citric acid concentration, was tested on two additional concentrations of citric acid (0.01 and 0.005 mM), due to a presumed hypersensitivity to citric acid and was therefore excluded from the two-way ANOVA for citric acid detection testing.

One-tailed binomial distribution tests were used to analyze performance on water control tests to ensure that performance was at the expected chance level (i.e., 50% correct responses) for each rat. A $p = 0.05$ was considered significant in all statistical tests.

RESULTS

Lesion Analysis

The behavioral analysis only included rats that met our criteria for complete lesions in GC. Thirteen BGCX and LGCX rats, and nine RGCX rats met that criterion. Of those rats, on average, BGCX lesions damaged 83% of GC and 91% of GC “core” (Figure 5), LGCX lesions damaged 89% of GC and 94% of GC “core” (Figure 6), and RGCX lesions damaged 91% of GC and 97% of GC “core” (Figure 7). In many of these cases, lesions extended beyond the GC into the claustrum, ventral to GC, dorsal to GC in GI, and occasionally into somatosensory areas.

In the same figures, there are overlap maps of the incomplete lesions of rats whose corresponding behavior was not included in the analysis. Of those rats, on average, BGCX lesions damaged 14% of GC and 12% of GC “core” (Figure 5), LGCX lesions damaged 34% of GC and 34% of GC “core” (Figure 6), and RGCX lesions damaged 21% of GC and 19% of GC “core” (Figure 7). Incomplete lesions tended to be more anterior in insular cortex suggesting that the posterior infusion of ibotenic acid may have failed in some of these cases.

Assessment of Stimulus Control

Although many measures were taken to maintain stimulus control, there were some instances that raised concern for loss of stimulus control of behavior that should be discussed. One RGCX rat (Rat 82) and one SHAM rat (Rat 68) performed significantly different from chance on the water test (Figure 8; Phase 2: WATER TEST) after citric acid testing, but their scores were nevertheless quite low. Moreover, RGCX Rat 82 did reach chance performance during citric acid testing and had monotonically decreasing performance as a function of concentration throughout testing, so that rat was included in the analysis. The SHAM rat that did not pass the water test (Rat 68), along with another RGCX rat (Rat 73) that did not reach chance performance during citric acid testing, did not have monotonically decreasing performance as a function of concentration throughout citric acid testing and, therefore, were removed from the analysis for citric acid due to an apparent loss of stimulus control. One RGCX rat (Rat 41; Figure 8; Phase 1: WATER TEST 1) after NaCl testing, and one LGCX rat (Rat 26; Figure 8; Phase 1: WATER TEST 2) and one SHAM rat (Rat 7; Figure 8; Phase 1: WATER TEST 2) after Maltrin testing for Phase 1, did not perform at chance during the water test but nonetheless had low scores (RGCX Rat 41 = 63%; LGCX Rat 26 = 60%; SHAM Rat 7 = 62%) and these rats reached chance performance during testing on that stimulus and performed in a concentration-dependent manner for all stimuli, so they were included in the analyses.

Effects of GC Lesions on Taste Sensitivity

NaCl.—The final group sizes for NaCl sensitivity testing were: BGCX, $n=13$; LGCX, $n=13$; RGCX, $n=9$; SHAM, $n=16$. A two-way ANOVA revealed a main effect of surgery as well as a surgery $\times$ concentration interaction between SHAM and BGCX (Table 3 and Figure 9). A separate two-way ANOVA also found a surgery $\times$ concentration interaction between SHAM and RGCX groups (Table 3 and Figure 9). There were no significant interactions between SHAM and LGCX (Table 3 and Figure 9). Two-way ANOVAs did reveal a significant main effect of concentration, unsurprisingly, across surgical groups. The analysis of the parameters of the individual animal curve fits also confirmed that there was a significant rightward shift in the NaCl taste sensitivity functions for the BGCX and RGCX groups relative to the SHAM animals. The EC_{50} (c -value) which represents the midpoint of the concentration-performance curve (i.e., taste sensitivity curve) and is operationally defined as the detection threshold, shifted to the right by $0.50 \log_{10}$ units on average in the BGCX group and by $0.39 \log_{10}$ in the RGCX group relative to the mean EC_{50} for the SHAM rats (Figure 10; Tables 4 and 5). There was no effect of lesion on slope (b), but there was an effect on asymptote (a) between SHAM and RGCX groups (tables 4 and 5); as can be seen in Figure 9, this effect was actually due to the RGCX group performing slightly better than the SHAM rats at the highest concentration. Animals with either complete bilateral or right hemisphere lesions in the GC were most affected compared to rats with incomplete lesions (Figure 9). As in our previous findings (Blonde et al., 2015), complete bilateral GC lesions affected NaCl detection, and, interestingly, the rats with large right hemisphere lesions in GC also showed impairment.

Maltrin.—The final group sizes for Maltrin sensitivity testing were: BGCX, $n=13$; LGCX, $n=13$; RGCX, $n=9$; SHAM, $n=16$. Two-way ANOVAs showed, as expected, main effects of concentration for each surgical group compared to the SHAM group, and there was a surgery $\times$ concentration interaction between the SHAM and RGCX groups (Table 3). Notwithstanding this interaction, Figure 9 clearly shows that the lesions had little effect on Maltrin taste sensitivity in the groups with lesions compared to SHAM rats. The analysis of the curve fit parameters between the surgical groups also showed no significant differences for asymptote, slope, or the EC_{50} (Figure 10, Table 5). Thus, the GC does not appear to be necessary for normal Maltrin detection.

Citric Acid.—Due to loss of stimulus control (see Assessment of Stimulus Control), one RGCX and one SHAM rat were removed from the analysis. The final group sizes for citric acid sensitivity testing were: BGCX, $n=13$; LGCX, $n=13$; RGCX, $n=8$; SHAM, $n=15$. A t -test of the c -values showed no shift in sensitivity (Figure 10, Table 5); however, a two-way ANOVA revealed a significant interaction of surgery and concentration between SHAM and BGCX rats, which appeared to be driven primarily by the differences in performance at the highest concentrations of citric acid (Table 3, Figure 9). Although there was no effect on the EC_{50} , asymptote, or slope of the psychometric functions (Table 5, Figures 9, and 10), bilateral GC damage appeared to have some effects on performance at two of the higher citric acid concentrations (t -tests, 10 mM citric acid: $p=0.001$; 5 mM citric acid: $p=0.002$). However, there were no statistical differences in performance to citric acid between SHAM and either unilateral lesion group.

NaCl Post-Detection Test.—The group sizes for the NaCl post-detection testing that occurred after citric acid testing for Phase 2 were: BGCX, $n=9$; LGCX, $n=7$; RGCX, $n=8$; SHAM, $n=8$. These rats were not only assessed for the psychometric NaCl taste functions at the start of the experiment but were also assessed at the end of the experiment for their performance to two concentrations tested, 0.0125 M and 0.003 M NaCl, along the dynamic range of the initial sensitivity curve (Table 6). Two-way ANOVAs (surgery $\times$ concentration) of performance to these two concentrations during the initial NaCl detection and the post-detection test revealed a significant main effect of surgery between BGCX and SHAM (see Tables 3 and 7). Interestingly, although there was a statistically significant difference between RGCX and SHAM during initial NaCl detection testing, that difference failed to reach the statistical rejection criterion for the post-detection NaCl test (see Tables 3 and 7); however, this was due to reduced performance in the SHAM group.

DISCUSSION

One of the most fundamental functions of any sensory system is the detection of stimuli of innate or learned relevance serving to optimize adaptive behavior under variable environmental conditions. In the context of the experiments here, rats learned to detect specifically trained taste stimuli to remediate thirst caused by overnight fluid deprivation. The question addressed centered on whether the primary taste cortex, a high-order site along the gustatory pathway, was necessary for the maintenance of this important basic sensory function. As we have seen before, rats with massive bilateral lesions in GC, confirmed and quantified objectively with our lesion mapping system (Schier et al., 2014; Schier et al., 2016), displayed significantly impaired taste sensitivity (i.e., rightward shifts of the EC_{50}) to some stimuli but not others.

In the case of NaCl, there was a substantial and significant 0.50 $\log_{10}$ unit increase in the EC_{50} (operationally defined as threshold) of the NaCl concentration-performance curve of the BGCX group. This replicated our previous finding with this salt as well as with KCl (Blonde et al., 2015; Bales et al., 2015). In the case of citric acid, no significant shifts in threshold, as assessed by the EC_{50} , were observed in the BGCX group, but performance was lower at some of the higher concentrations. We cannot rule out that, at these higher concentrations, trigeminal input originating from the acid stimulation of the oral cavity contributed to the overall perception of the signal. If so, the performance deficit at some of the higher citric acid concentrations in the BGCX group may have arisen from the fact that the lesions encroached on GI, where oral somatosensory cortex is situated (Kosar et al., 1986a). At lower concentrations, the performance may have been strictly guided by taste and not dependent on the presence of GI, and, in the case of citric acid, not dependent on GC either.

In the case of Maltrin, a highly preferred maltodextrin stimulus to rodents, performance was unperturbed by the extensive bilateral lesions of GC and only weakly impaired by RGCX lesions, with no significant shift in the EC_{50} in either lesion group. We have previously shown that sucrose sensitivity is also entirely unaffected by this same lesion (Bales et al., 2015) and our results with Maltrin extend this finding by showing the same outcome with a different normally preferred stimulus that is thought to engender a perceptual quality

(oligosaccharide taste) distinct from that associated with sugar (e.g., “sweetness”) (Nissenbaum and Sclafani, 1987; Sclafani, 2004). Although both stimuli contain calories and have the possibility of stimulating postingestive receptor mechanisms to guide the performance of the animals, we find this highly unlikely. On a given trial, the animals receive a maximum of 50 μ L of fluid in 3 s and then have up to an additional 5 s to respond (but most animals respond immediately on most trials). Thus, it is difficult to believe that the sensory systems of the esophagus and stomach would be adequately sensitive to be able to discriminate the chemical composition of the fluid on a given trial to sufficiently inform a choice.

Collectively across our studies (Blonde et al., 2015; Bales et al., 2015), it is clear the effects of extensive bilateral lesions of the GC on taste sensitivity are chemospecific. The detection of certain classes of taste compounds such as salts are substantially impacted by the lesions, whereas sensitivity to other classes, such as preferred carbohydrate stimuli, appears to be impervious to the absence of the GC. The detection of quinine and citric acid are moderately and modestly impaired, respectively, by GCX. Such a profile of results suggests that some forms of basic sensory-discriminative taste function are not uniformly dependent on the same brain regions in the gustatory neuraxis. It remains to be determined whether even larger insular cortex lesions would have led to impairments in sensitivity to sucrose and Maltrin, but the lesions encompassed a very expansive majority of the conventionally defined GC and we went to great lengths to objectively quantify the extent, location, and symmetry of the neurotoxin-induced damage. Nevertheless, the fact remains that disruption of taste sensitivity to a given class of taste compounds due to a neural manipulation which is restricted to a specific brain site, such as a lesion, does not guarantee that similar impairments will be evident with other classes of taste stimuli.

The taste detection task used here is more complex than it may at first appear. It involves learning, memory, motivation, and decision making, all at the limens of sensibility. Given the size of the lesions, it is truly remarkable that performance to some stimuli is absolutely normal. Even in the case of salts, one has to be impressed by the ability of animals to perform the task quite well at higher concentrations. Thus, these lesions do not cause general ageusia. Clearly, the remaining, likely subcortical, portions of the gustatory system are sufficient to support this function. It remains an open question how much of the gustatory system is necessary to allow an animal to perform a basic detection task whether normally with low concentrations or abnormally with high concentrations. Although lesions of the gustatory zone of the PBN do not completely render all rats aguesic, such neural damage at this level of the gustatory neuraxis does severely impair taste sensitivity to sucrose and NaCl (Spector et al., 1995). Accordingly, it would appear that at least the gustatory PBN and/or its rostral targets are necessary for the maintenance of normal taste detection. It would be instructive to silence those forebrain targets singly or in combination and then psychophysically assess the consequences on taste detection with a theoretically relevant panel of chemical stimuli.

Rat GC seems to be different than mouse GC with respect to its necessity to support sensory-discriminative function. In mice, the pharmacological silencing of the anterior GC disrupts performance to sucrose, but not to quinine, when these stimuli are used as cues in a

Go/No-Go task. Whereas the pharmacological silencing of the posterior GC disrupts performance to quinine, but not sucrose in the same task (Peng et al., 2015). The species difference remains to be understood, but might be due to the means of silencing GC (lesions vs. pharmacological), the extent or precise location of the cortical area manipulated, the specific nature of the discrimination task, or the properties of the neural circuit organization in insular cortex such as the potential presence of a more explicit chemotopy in the mouse (Chen et al., 2011; Fletcher et al., 2017) compared with the rat (Yamamoto et al., 1985; Accolla et al., 2007). Nevertheless, the mouse findings buttress the principle that sensory-discriminative taste function in a given brain site can be chemospecific.

Whether or not a potential chemotopy in GC exists symmetrically in both hemispheres has yet to be explored. Surgical limitations of GC imaging studies only allow measurements from one hemisphere per rodent (Accolla et al., 2007: right hemisphere; Chen et al., 2011: not reported; Fletcher et al., 2011: not reported). Lateralization in rodent taste behavior has received little attention. Although Schier et al. (2016) found unilateral lesions in posterior left or right rat GC did not affect the retention of a CTA, the effects of unilateral GC damage on taste detection had not been assessed in rodents. Here, the initial deficit of NaCl taste detection in RGCX rats was in line with Small et al. (1997) showing humans with lesions in their right anterior temporal lobe were impaired on a citric acid taste recognition task and in opposition to findings that the greatest taste impairments to NaCl was found in humans with left GC damage (Stevenson et al., 2013). The functions of brain regions from left to right are not always symmetrical between hemispheres (see Alqadah et al., 2018), and this may be the case with regards to NaCl taste detection in the rat GC. Given the paucity of studies focused on the taste-related behavioral effects of unilateral lesions in the rat, it would be prudent to explore the potential for left vs. right hemisphere differences in their contribution to taste detection by extending the stimulus array to not only include NaCl, but other salts and quinine as well, the sensitivities to which are known to be affected by bilateral GC damage.

In summary, in the rat, the GC is necessary for normal taste detection of some taste compounds but not all. It appears to be unnecessary for the maintenance of normal affective responsiveness to preferred and avoided stimuli as measured by intake and preference tests (Braun et al., 1982; Dunn and Everitt, 1988; Benjamin and Pfaffmann, 1955; but see Benjamin and Ackert, 1959), brief access assays (Hashimoto and Spector, 2014), and taste reactivity (King et al., 2015). An intact GC, specifically the posterior portion, is necessary for the normal acquisition and expression of a CTA (Schier et al., 2014; Schier et al., 2016). This suggests that GC may be critical in more complex processes that includes integration of taste signals with those from other modalities so as to optimize adaptive taste-related behavior such as what occurs during associative learning (Vincis and Fontanini, 2016). Moreover, GC may be involved with the integration of chemosensory signals leading to flavor perception, as suggested by the identification of single neurons in this brain site that respond to both taste and olfactory stimuli (Samuelson and Fontanini, 2017; Maier, 2017) and recently complemented by the finding that retronasally, but not orthonasally, learned preferences requires the GC (Blankenship et al., 2019). Nevertheless, our findings demonstrate that the detection of different classes of taste compounds is not uniformly processed in gustatory brain regions. Future work can explore exactly what areas are

necessary and sufficient to maintain normal detectability for an array of taste stimuli that differ in fundamental ways (e.g., quality, hedonics, caloric).

Acknowledgements:

The authors would like to acknowledge the advice and technical assistance of Ginger Blonde. We also would like to thank Fabienne Schmid for her assistance in the behavioral testing of the rats. This work was conducted in partial fulfillment of doctoral degree at Florida State University. Michelle Bales is currently in the Department of Molecular Biophysics and Physiology, Vanderbilt University, Nashville, TN, United States. Research reported in this publication was supported in part by the National Institute of Deafness and Other Communication Disorders under award number R01-DC009821 (ACS) and FSU Chemical Senses Training (CTP) Grant Award T32-DC000044 (MBB). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 1.
Trial structure.

Figure 2.

Schematic drawing from Schier et al., (2016), displaying a lateral view of the insular cortex (IC), subdivided into three AP regions (right), together with four representative coronal sections of IC (left). The approximated location of conventionally defined gustatory cortex (GC) and visceral cortex (VC) are indicated with blue and red hatching, respectively. The approximate AP borders of these three regions are indicated by the vertical lines in the lateral surface view. Lesion damage in IC and surrounding areas were graphically represented by a two-dimensional (2-D) mapping grid and quantified. A detailed description of the construction of these maps is presented in Materials and Methods. Briefly, as shown on the 1.2 mm AP plate, over the grid constitutes the area of interest parceled into five major dorsal/ventral (DV) subsections and five major medial/lateral (ML) subsections. As shown on the left, the DV subsections are represented by the five major columns of the 2-D mapping grid, and the ML subsections are represented by the five subcolumns within each of the DV columns. An observer blinded to the performance of the rat assessed the tissue represented by each cell of the grid for damage. If the lesion encompassed less than half of the tissue, then a score of 0 was assigned for that cell. If the lesion encompassed more than half of the tissue, a score of 0.5 was assigned for that cell, and if the lesion encompassed all of the tissue, a score of 1.0 was assigned for that cell. These criteria were used to assess

successive serial 50 μm coronal sections, represented by rows on the 2-D grid, for damage. AI, agranular insular cortex; AP, anterior/posterior; D, dorsal; DI, dysgranular insular cortex; GI, granular insular cortex; IC₁, region of insular cortex containing the anterior gustatory cortex; IC₂, region of insular cortex containing the posterior gustatory and anterior visceral cortex; IC₃, region of insular cortex containing the posterior visceral cortex; L, lateral; M, medial; rf, rhinal fissure; V, ventral.

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Figure 3. Representative bilateral lesion maps. The left hemisphere (left panel), right hemisphere (right panel), and symmetry maps (center panel) include major columns representing the region dorsal to the insular cortex (D), the granular insular cortex (GI), the dysgranular insular cortex (DI); agranular insular cortex above the rhinal fissure (AI) and the region ventral to the rhinal fissure (V). The major columns are further divided into five subcolumns representing the medial to lateral portions as described in Figure 2. The order of the major columns and subcolumns for the map of the right hemisphere is the mirror image of that shown for the map of the left hemisphere. The symmetry maps adopt the column order shown for the left hemisphere. Top: photomicrographs and scoring of a representative large bilateral lesion of a rat whose lesion met the inclusion criteria for behavioral analyses (>50%

of GC, and >70% of the GC “core”, containing a lesion). Bottom: photomicrographs and scoring for a representative small bilateral lesion in a rat that did not meet the inclusion criteria, and was therefore not included in the behavioral analyses. Both left and right hemispheres are shown for each representative map with the resulting symmetry map (middle panel). Complete lesion to an area (one grid cell) is shown in red and received a score of 1.0. Damage that was less than complete but at least half within the area is indicated in orange and was given a score of 0.5. Less than half of the area with damage, is shown in white and was given a score of 0. The AP boundaries of traditionally defined GC are represented by the horizontal solid lines at 2.3 and 0.2 mm (AP relative to bregma). Medium-dashed horizontal lines show anterior and posterior boundaries of GC “core” at 1.8 and 0.6 mm (AP relative to bregma). The approximate center of GC is shown with short-dashed lines at 1.2 mm (AP relative to bregma). Photomicrographs of the representative lesion maps are indicated with labeled coordinates (in mm, relative to bregma) for each hemisphere. Scale bar = 1 mm.

Figure 4. Representative unilateral lesion maps. Top (left): photomicrographs and scoring for a representative large lesion in the left hemisphere; top (right) photomicrographs and scoring for a representative large lesion in the right hemisphere. Bottom (left) photomicrographs and scoring for a representative small lesion in the left hemisphere; bottom (right) photomicrographs and scoring for a representative small lesion in the right hemisphere. See Figures 2 & 3 for details.

Figure 5. Overlap maps of large and small bilateral lesions. Compiled symmetry maps of rats meeting lesion criteria for behavioral analyses (left panel; 50% of GC and 70% of the GC “core” containing a lesion; n = 13) and lesions that did not meet criteria and were not included in behavioral analyses (right panel; n = 12). The lesions in the included rats encompassed 83% of GC and 91% of GC “core” on average. The lesions for the excluded rats covered 14% of GC and 12% of GC “core”. The color key that shows the average lesion score for the group in each cell by color is between the overlap maps. D: Dorsal to granular insular cortex. GI: Granular insular cortex. DI: Dysgranular insular cortex. AI: Agranular insular cortex, dorsal to the rhinal fissure. V: Ventral to the rhinal fissure. L: Lateral. M: Medial. Solid lines show anterior and posterior boundaries of the traditionally defined GC at 2.3 and 0.2 mm (AP

relative to bregma). Medium-dashed lines indicate anterior and posterior boundaries of GC “core” at 1.8 and 0.6 mm (AP relative to bregma). Short-dashed lines indicate the approximate center of GC at 1.2 mm (AP relative to bregma).

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Figure 6. Overlap maps of large and small left hemisphere lesions. Compiled Maps of rats meeting lesion criteria for behavioral analyses (left panel; 50% of GC and 70% of the GC “core” containing a lesion; n = 13) and lesions that did not meet criteria and were not included in behavioral analyses (right panel; n = 6). The lesions for the included rats encompassed 89% of GC and 94% of GC “core” on average. The lesions for the excluded rats covered 34% of GC and 34% of GC “core”. See Figure 5 for a detailed description of the overlap maps.

Figure 7. Overlap maps of large and small right hemisphere lesions. Compiled Maps of rats meeting lesion criteria for behavioral analyses (left panel; 50% of GC and 70% of the GC “core” containing a lesion; n = 9) and lesions that did not meet criteria and were not included in behavioral analyses (right panel; n = 12). The lesions for the included rats encompassed 91% of GC and 97% of GC “core” on average. The lesions for the excluded rats covered 21% of GC and 19% of GC “core”. See Figure 5 for a detailed description of the overlap maps.

Figure 8.

Water tests. Performance from chance (50%) on Water Test days. All arrows point to rats that performed above chance according to a one-tailed binomial distribution test. Top (left), Phase 1: WATER TEST 1 after NaCl detection testing; arrow points to Rat 41 performance. Top (right), Phase 1: WATER TEST 2 after Maltrin detection testing; arrows point to the performance of Rat 7 and Rat 26. Bottom (left), Phase 1: WATER TEST 3 after citric acid detection testing. Top (right), Phase 2: WATER TEST after citric acid detection testing; arrows pointing to the performance of Rat 82 and Rat 68. See Assessment of Stimulus Control section for more detail.

Figure 9.

Mean ($\pm$ SE) proportion correct as a function of concentration for NaCl (top), Maltrin (center), and Citric Acid (bottom). Performance of the SHAM group was compared with that for the BGCX (left), LGCX (middle), and RGCX (right) groups. The curves were fit to the data based on a 3-parameter logistic function (see Data Analysis). SHAM (blue), BGCX (red), LGCX (green), and RGCX (purple). Significant results from separate surgery $\times$ concentration mixed two-way ANOVAs are shown in each panel. For more details on statistical outcomes see Table 3. BGCX had a very clear effect on performance to NaCl, shifting the sensitivity curve rightward (also see Figure 10), and had an effect on performance to citric acid, but in the latter case a lateral shift in the sensitivity curve was not statistically evident (see Figure 10). RGCX also significantly impaired performance to NaCl

but the lateral shift in the sensitivity curve was somewhat weaker than what was observed in the BGCX group (also see Figure 10). RGCX significantly impaired performance to some concentrations of Maltrin, but, as can be seen, the magnitude was weak and was not observed after BGCX.

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Figure 10.

Individual c -values (EC_{50}) for rats with histologically confirmed complete lesions tested with NaCl (top), Maltrin (middle), and citric acid (bottom). The SHAM group was compared with the BGCX (left), LGCX (center), and RGCX (right) groups. Means and $\pm$ SEs are represented by solid lines and dashed-lines respectively in each panel. An asterisk indicates a significant difference ($p < .05$). For more details on statistical outcomes see Table 5. The EC_{50} effectively measures lateral shifts in the taste sensitivity curves. Accordingly, in this analysis, only the BGCX and RGCX groups displayed significantly decreased taste sensitivity and only to NaCl.

Table 1.

Schedule of experimental phases.

Stage	Session Length	# of Sessions	Taste Stimuli
Surgery: Bilateral (BGCX), left unilateral (LGCX), right unilateral (RGCX) ibotenic acid lesions targeting GC and SHAM rats given PBS (SHAM) vehicle infusions			
Recovery		9–22	
Detection Thresholds:			
NaCl			
Lick Training	30 min	3–4 [†]	DW
Side Training	30 min	4	0.4 M NaCl or DW
Alternation	30 min	4	0.4 M NaCl or DW
Random Training I	30 min	2	0.4 M NaCl or DW
Random Training II	30 min	1–13 [‡]	0.4 M NaCl or DW
Testing	30 min	24–30 [‡]	NaCl [¶] and DW
Water Test	30 min	0–1 [§]	
Maltrin			
Training II	30 min	4–6 [†]	20% Maltrin and DW
Testing	30 min	21–27 [‡]	Maltrin [¶] and DW
Water Test	30 min	0–1 [§]	DW
Citric Acid			
Training II	30 min	5–6	30, 20, or 10 mM Citric Acid and DW
Testing	30 min	27–32 [‡]	Citric Acid [¶] and DW
Water Test	30 min	1	DW
Post-Detection Threshold Testing			
Testing	30 min	4	0.0125 M, 0.003 M NaCl and DW

[†]Ranges were given when animals did not reach criteria to progress to the next training stage.

[‡]Range indicates animals did not perform 70% on the control day prior to a test day, so that test day concentration was repeated and the data for both days were averaged.

[§]A range is reported because a Water Test was not given (see Methods Section for detail).

[¶]One concentration was tested per session.

Table 2.

Anatomical landmarks based on the rat brain atlas (Paxinos and Watson, 2007).

Anteroposterior Atlas Coordinate (mm relative to β)	Anatomical Description
4.2	The section right before layer 1a of piriform cortex (Pir1a) separates from the midline
2.76	Striatum (Cpu) becomes visible in the middle of corpus collosum
2.3	Corpus collosum from both hemispheres meet at the midline: genu of the corpus collosum (gcc)
1.56	Indusium griseum (IG) disappears
0.72	Anterior commissure (aca) is no longer lateral to Lateral Ventricle (LV)
0	One section before aca meets at the midline (approximately at bregma)
-0.48	Bed nucleus (BAC) is most prominent and aca is still bridging the two hemispheres
-1.72	CA3 field of the hippocampus becomes visible

Modified from Bales et al., 2015.

Table 3.

Two-way ANOVAs for detection testing.

NaCl	Surgery	Concentration	Surgery × Concentration
BGCX vs. SHAM	F(1, 27)=6.325, p=0.018	F(11, 297)=184.770, p<0.001	F(11, 297)=4.703, p<0.001
LGCX vs. SHAM	F(1, 27)=1.715, p=0.201	F(11, 297)=188.456, p<0.001	F(11, 297)=1.081, p=0.376
RGCX vs. SHAM	F(1, 23)=0.122, p=0.122	F(11, 253)=189.230, p<0.001	F(11, 253)=5.217, p<0.001

Maltrin	Surgery	Concentration	Surgery × Concentration
BGCX vs. SHAM	F(1, 27)=0.141, p=0.710	F(9,243)=151.232, p<0.001	F(9,243)=1.458, p=0.164
LGCX vs. SHAM	F(1, 27)=0.396, p=0.534	F(9, 243)=171.992, p<0.001	F(9, 243)=1.227, p=0.279
RGCX vs. SHAM	F(1, 23)=1.165, p=0.292	F(9, 207)=116.285, p<0.001	F(9, 207)=2.064, p=0.034

Citric Acid	Surgery	Concentration	Surgery × Concentration
BGCX vs. SHAM	F(1, 26)=1.086, p=0.307	F(10, 260)=107.209, p<0.001	F(10, 260)=3.543, p<0.001
LGCX vs. SHAM	F(1, 26)=0.648, p=0.428	F(10, 260)=143.500, p<0.001	F(10, 260)=1.345, p=0.207
RGCX vs. SHAM	F(1, 21)=0.013, p=0.912	F(10, 210)=105.239, p<0.001	F(10, 210)=0.455, p=0.917

Table 4.

Mean (SE) of curve parameters for detection testing within surgical groups.

NaCl	a	b	c[†]
SHAM	0.94 (0.03)	-1.30 (0.48)	-2.64 (0.32)
BGCX	0.97 (0.06)	-1.14 (0.46)	-2.14 (0.41)
LGCX	0.95 (0.04)	-1.51 (1.57)	-2.43 (0.39)
RGCX	0.97 (0.02)	-1.61 (1.52)	-2.25 (0.32)

Maltrin	a	b	c[†]
SHAM	0.96 (0.04)	-1.96 (1.33)	0.37 (0.22)
BGCX	0.95 (0.06)	-1.47 (0.90)	0.28 (0.41)
LGCX	0.98 (0.03)	-1.49 (0.73)	0.31 (0.36)
RGCX	0.97 (0.03)	-2.15 (1.35)	0.53 (0.33)

Citric Acid	a	b	c[‡]
SHAM	0.96 (0.05)	-1.61 (1.27)	0.31 (0.27)
BGCX	0.97 (0.06)	-1.44 (1.17)	0.49 (0.60)
LGCX	0.97 (0.03)	-1.51 (1.19)	0.24 (0.43)
RGCX	0.98 (0.03)	-1.17 (0.48)	0.38 (0.40)

[†]Values in log₁₀ (M) concentrations

[‡]Values in log₁₀ (mM) concentrations

Table 5.

T-tests for curve parameters for detection testing within surgical groups.

NaCl	a	b	c
SHAM vs. BGCX	t(27)=1.821, p=0.080	t(27)=0.901, p=0.376	t(27)=3.652, p=0.001 *
SHAM vs. LGCX	t(27)=0.902, p=0.375	t(27)=-0.517, p=0.609	t(27)=1.558, p=0.131
SHAM vs. RGCX	t(23)=3.004, p=0.006 *	t(23)=-0.784, p=0.441	t(23)=2.893, p=0.008 *

Maltrin	a	b	c
SHAM vs. BGCX	t(27)=-0.44, p=0.67	t(27)=1.12, p=0.273	t(27)=-0.748, p=0.461
SHAM vs. LGCX	t(27)=1.697, p=0.101	t(27)=1.117, p=0.274	t(27)=-0.562, p=0.579
SHAM vs. RGCX	t(22)=0.525, p=0.605	t(22)=-0.326, p=0.747	t(23)=1.464, p=0.157

Citric Acid	a	b	c
SHAM vs. BGCX	t(26)=0.319, p=0.753	t(26)=0.352, p=0.727	t(26)=1.080, p=0.290
SHAM vs. LGCX	t(26)=0.826, p=0.417	t(26)=0.205, p=0.839	t(26)=-0.531, p=0.600
SHAM vs. RGCX	t(21)=0.795, p=0.435	t(21)=0.920, p=0.368	t(21)=0.505, p=0.619

* Significant effects/interactions even after Bonferroni correction.

Table 6.

Mean (SE) of NaCl performance for 0.0125 M and 0.003 M NaCl testing for Phase 2.

NaCl	During Detection		Post-Detection	
	0.0125 M	0.003 M	0.0125 M	0.003 M
SHAM	0.84 (0.02)	0.77 (0.03)	0.80 (0.04)	0.70 (0.03)
BGCX	0.75 (0.03)	0.66 (0.04)	0.70 (0.04)	0.60 (0.03)
LGCX	0.83 (0.02)	0.68 (0.03)	0.74 (0.03)	0.62 (0.02)
RGCX	0.78 (0.04)	0.63 (0.03)	0.73 (0.03)	0.65 (0.02)

Table 7.

Two-way ANOVAs for 0.0125 M and 0.003 M NaCl testing for Phase 2.

During Detection	Surgery	Concentration	Surgery × Concentration
BGCX vs. SHAM	F(1, 15)=7.601, p=0.015 *	F(1, 15)=47.333, p<0.001 *	F(1, 15)=0.186, p=0.672
LGCX vs. SHAM	F(1, 13)=1.496, p=0.243	F(1, 13)=46.332, p<0.001 *	F(1, 13)=.061, p=0.322
RGCX vs. SHAM	F(1, 14)=7.982, p=0.013 *	F(1, 14)=32.737, p<0.001 *	F(1, 14)=3.267, p=0.092

Post-Detection	Surgery	Concentration	Surgery × Concentration
BGCX vs. SHAM	F(1, 15)=5.611, p=0.032 *	F(1, 15)=18.367, p=0.001 *	F(1, 15)=0.011, p=0.919
LGCX vs. SHAM	F(1, 13)=2.693, p=0.125	F(1, 13)=34.039, p<0.001 *	F(1, 13)=0.113, p=0.742
RGCX vs. SHAM	F(1, 14)=2.435, p=0.141	F(1, 14)=24.366, p<0.001 *	F(1, 14)=0.255, p=0.621

* Significant effects/interactions