Learned aversions in rats lacking gustatory neocortex: Truly aversive or simply avoided?

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Missy R. Orr

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Converging evidence from anatomical, behavioral, and electrophysiological studies have identified the cortical region of the rat brain involved with the processing of taste information. The gustatory neocortex (GN) lies on the anterolateral surface of the brain adjacent to the rhinal sulcus and is bisected by the middle cerebral artery.

The gustatory pathway in mammals begins at the tongue. Information about taste is transmitted to the brain through three cranial nerves. Information coming into contact with the anterior part of the tongue travels through the facial nerve (VII). Receptors on the posterior part of the tongue send information via the IX cranial nerve, the glossopharyngeal nerve. The X cranial nerve (the vagus nerve) receives input from the epiglottis and palate. Once information has been transmitted to the various cranial nerves, it proceeds to the solitary nucleus (nucleus of the solitary tract) which is located in the medulla. From here, axons from taste sensitive neurons project forward to the parabrachial nucleus of the pons. Taste information travels from this second relay station to the thalamic taste area in the ventral posterior thalamic nucleus.

Some information is also projected to the lateral hypothalamus and limbic system. The thalamic projections then lead to the somatic facial region of the cortex.

Electrophysiological studies

Benjamin and Pfaffmann (1955) performed electrophysiological tests in which they electrically stimulated the chorda tympani, a branch of the VII nerve, and the IXth nerve and recorded the cortical potentials with an oscillograph (an ink-writing machine used to record evoked potentials). Results showed the chorda area to lie anterior to and overlap the IXth nerve area, with the chorda tympani being represented bilaterally, while the IXth nerve was found to be represented only contralaterally. Both areas were roughly 2 sq mm in size with the variability between animals being quite small.

Yamamoto, Matsuo, and Kawamura (1980) performed electrophysiological studies to determine the cortical gustatory region of the rat. Thirty male rats were used as subjects and the chorda tympani, glossopharyngeal nerve, and the lingual branch of the trigeminal nerve were electrically stimulated. These nerves were stimulated with approximately twice the required voltage to achieve a response. Results indicated that, compared to adjacent trigeminal somatosensory nuclei, the

subcortical gustatory areas were minuscule. An area less than 2 sq mm dorsal to the rhinal sulcus and bridging the middle cerebral artery constituted the entire site of responsiveness. The cortical projection area of the chorda tympani consisted of two separate areas, one lying anterior to the middle cerebral artery and the other posterior to the middle cerebral artery. Both were dorsal to the rhinal sulcus, with the former located just above it. Cortical projections from the lingual nerve fell in relatively the same area as those from the chorda tympani, with quite a bit of overlap. Projections from the glossopharyngeal nerve, however, were only received on one area of the cortex just posterior to the middle cerebral artery and dorsal to the rhinal sulcus. All cortical projections were represented bilaterally.

The above electrophysiological studies, along with others by Ganchrow and Erickson (1972), Norgren and Wolf (1975), Yamamoto and Kawamura (1972), and Yamamoto, Yuyama, and Kawamura (1981), indicate that cortical neurons within the chorda tympani area respond to taste, temperature, and touch. Neurons responding specifically to taste appear to be located at the ventral and posterior portion of the taste nerve area (Benjamin & Pfaffmann, 1955).

Behavioral studies

In light of the results from the electrophysiological studies, researchers felt compelled to define further and more explicitly the taste nerve area. Benjamin and Pfaffmann (1955) performed behavioral studies to localize the gustatory neocortex area in rats. Their main focus was on the IXth and chorda tympani nerve area. Their study began with two-bottle quinine preference tests on a group of 15 male rats. Rats were tested with various quinine solutions to determine a "threshold" concentration (a concentration at which rats would consume 25% of their total fluid intake); 50% consumption indicated inability to discriminate the quinine from water. Neocortex lesions were then performed on the rats; five rats underwent experimental surgery in which all of the critical area was removed bilaterally; five rats underwent control surgery, where other parts of the neocortex (excluding the composite gustatory area) were removed; and five rats remained intact to test for normal threshold deviation with repeated testing. Animals were postoperatively tested for quinine consumption to determine a threshold. Postoperative thresholds for all five experimental rats increased, while both control groups maintained their thresholds at a relatively constant level. Benjamin and Pfaffmann concluded that bilateral damage to the

composite IXth and chorda tympani nerve area produced permanent deficits in quinine discrimination in a two-bottle preference situation and that removal of the rest of the neocortex had no measurable effect (Benjamin & Pfaffmann, 1955).

Benjamin and Akert (1959) expanded the study of the cortical taste area by including more types of cortical lesions. The three types of lesions employed were: unilateral lesions varying in size and locus; classical bilateral symmetrical ablation; and complete decortication except one unilateral circumscribed island to define the minimum amount of cortex necessary for function ("islet preparation"). Results showed that animals with complete bilateral ablations of the "taste nerve area" had postoperative thresholds up to eight times that of their preoperative threshold; no lesions outside this area produced impairment. Even completely neodecorticated animals that had only the taste nerve area unilaterally intact were able to discriminate normally.

Braun, Lasiter, and Kiefer (1982) described several taste tests involving normal rats and GN rats to determine if there were any threshold and/or preference differences between the two types of rats. One-bottle tests measuring sucrose, quinine hydrochloride, sodium hydrochloride, and hydrochloric acid consumption showed

virtually no difference in discrimination between the control and experimental groups. GN rats were slightly hyperresponsive to moderate and high concentrations of sucrose and sodium chloride, resulting in a higher rejection threshold, but this did not suggest changes in detection thresholds for any of the taste stimuli. Replication of the sucrose test 3 months after the aforementioned test yielded virtually the same responsiveness but with lower rejection thresholds and somewhat less potent responsiveness to moderate concentrations. Replications using lower concentrations of the four tastants failed to indicate differences in consumption between the control and GN group. In summary, Braun et al. found that GN rats have essentially normal taste thresholds when compared with normal rats. Therefore, the GN does not appear to be critical for taste detection or taste acceptance and rejection in simple preference tests.

Review of the above studies reveal the cortical gustatory area to be an approximately 1 x 3 mm area just above the rhinal sulcus and bisected by the middle cerebral artery. The area crucial to taste discrimination is comprised of the anterior chorda tympani area and the ventral third of the glossopharyngeal nerve area.

Associative Taste Processes of the GN

Benjamin and Akert (1959) summarized that the "taste nerve area" was the region of the brain particularly related to afferent information from the tongue. However, recent studies have gone on to expand the definition of this area as being significantly involved in associative taste processes. Furthermore, the associative importance of the GN area seems to be specifically related to gustatory input. As pointed out by Leach in 1978, rats trained to avoid both an odor and a taste cue (separately administered) would retain the odor habit but not the taste habit after GN ablation (Kiefer, Leach, & Braun, 1984). Thus, there appears to be an abnormal gap between taste detection and associative prominence of taste cues in GN rats. Braun, Kiefer, and Ouellet (1981) suggested that dissociation between simple detection and memorial recognition functions is a general effect of damage to the sensory neocortical region in rats. Despite this division, they went on to show that the GN rats could be retrained to avoid the taste cue.

One form of taste associations is that of conditioned taste aversions. Taste aversions in rats are easily formed because rats are naturally neophobic. Upon encountering a new food item, a rat will approach it with caution, neck outstretched, nose and vibrissae

twitching. If the rat becomes ill after sampling this new food (stomach retches, gaping, and chin rubbing), the rat will avoid this food on future encounters. Thus, it appears evident that internal malaise sets the stage for food aversion learning. Lett (1985) performed a study to support this hypothesis. Lett used a distinctive taste and a distinctive place and experimented with three different drugs: gallamine, naloxone, and lithium chloride. Gallamine and naloxone cause neuromuscular impairment, while lithium chloride induces an emetic reaction. The results were distinct place avoidance in conjunction with the gallamine and naloxone, but weak taste aversions; strong taste aversions were produced with lithium chloride, but weak place aversions. Similar studies testing the effectiveness of other noxious stimuli in creating taste aversions have been done. For example, Hankins, Garcia, and Rusiniak (1974) reported that a sweet flavor followed by a punishing electrocutaneous shock did not result in the flavor becoming distasteful to the rat.

Normal rats readily learn aversions to a novel taste stimulus when it is followed, sometimes as much as 48 hours later, by an aversive unconditioned stimulus (e.g., lithium chloride, cyclophosphamide). Rats that have learned such an association will, upon future presentations of the stimulus, consume little or none of

it. Furthermore, rats have been shown to discriminate the paired taste from other tastes and therefore selectively reject it (Kiefer & Braun, 1979; Lorden, 1976; Lorden, Kenfield, & Braun, 1970).

The gustatory neocortex has been proven to play a role in the acquisition of learned taste aversions. It has been found that if an animal forms a taste aversion to a novel taste and is subsequently given bilateral GN ablations, the animal no longer retains that acquired aversion; the animal behaves as a naive animal would (Braun et al., 1981; Yamamoto, et al., 1980).

Effects of GN Lesions on Conditioned Taste Aversions in Rats

Rats lacking GN seem unable to form single trial taste aversions regardless of taste categories (sweet, bitter, salty). Braun et al. (1982) concluded that GN ablations in rats interfere with subtle taste discriminations, but do not interfere with basic taste reactivity. Rather, it appears that the GN area is more involved in memorial taste processes; rats lacking GN tend to respond to both familiar and novel tastes as normal rats respond to familiar tastes (Kiefer & Braun, 1977). Various studies show taste stimuli such as .153 M sodium chloride, .146 M sucrose solutions (Kiefer & Braun, 1979), and .0041 M sodium saccharide (Braun, Slick, and Lorden, 1972), when paired with an illness

producing drug such as lithium chloride, apomorphine, or cyclophosphamide, did not result in acquired taste aversions for GN rats.

The effect of GN lesions on the ability of rats to learn taste aversions is most striking when the taste paired with illness is a sweet cue; however, the lesion appears to affect taste in general (Lorden, 1976). Braun et al. (1972) found that both normal rats and GN rats behaved in a similar manner in quinine aversion tests. Both groups of rats were trained to avoid either quinine or saccharin by receiving an injection of cyclophosphamide following consumption of one of the solutions. Immediately after acquisition of the taste aversion, rats were tested for consumption of the paired solution. Both groups showed significant rejection of the quinine solution, but the GN rats in the saccharin test did not develop an aversion to the saccharin cue. Lorden reported similar results in Experiments 1-3 of her 1976 study.

Lorden (1976) did further studies pairing quinine and hydrochloric acid with drug-induced illness in GN rats. Both groups of trained rats suppressed intake of their respectively trained taste. However, Lorden also found that the GN-quinine group generalized its suppression to the hydrochloric acid following the quinine-drug

pairing. Similarly, the GN-acid group suppressed its quinine consumption following the acid-drug pairing. Thus, the aversions displayed were not as discriminatively specific in the GN rats as compared to the normal rats.

Rats with bilateral GN ablations show deficits in acquiring illness-induced aversions (Braun et al., 1972). However, repeated trials using lithium chloride as the unconditioned stimulus eventually produced significant aversions to .0041 M saccharin in one study (Hankins et al., 1974). Thus, it appears that GN rats may be capable of learning to avoid taste stimuli under certain conditions.

Kiefer and Braun (1979) paired either .146 M sucrose or .153 M sodium chloride with apomorphine injections over a period of 5 training trials using a repeated trials design. Rats lacking GN and normal rats were compared to control groups of rats that were injected with physiological saline following either the sucrose or sodium chloride solution. Results indicated that both normal rats and GN rats receiving tastes paired with drug injection significantly reduced consumption of their respective tastant as compared to the saline-injected control groups. The conditioned aversions were discriminatively specific in that the rats markedly reduced consumption of the taste that had been paired

with drug injection as compared to the unpaired taste. It is important to note however, that the GN rats acquired taste aversions more slowly than the normal rats and that the difference in consumption between paired and unpaired tastes by the GN rats was not as great as that seen in normal rats.

Other parameters of taste aversion learning experiments can be manipulated to produce an apparent aversion in a GN rat. Lorden (1976) increased a sucrose solution to 1.0 M concentration and then did single trial, drug-induced toxicosis tests on both normal and GN rats. Normal rats consumed no sucrose following sucrose-drug pairings, and GN rats consumed considerably less sucrose than did their yoked control counterparts. Thus, at relatively high stimulus concentrations, GN lesioned rats exhibited aversions to sweet cues, although the aversions were still weaker than those seen in normal rats.

Hankins et al. (1974) found it possible to train GN rats to avoid a saccharin solution with multiple pairings of the tastant and lithium chloride. Over the course of 10 days, the experimenters paired a saccharin solution three times with a lithium chloride intubation. They used two different concentrations of saccharin and followed them either immediately (5 min) or after a delay (30 min) with the lithium chloride treatment.

Rats with GN lesions learned, albeit slower than their normal counterparts over the three presentation design, to associate the sweet taste with illness when illness immediately followed. The GN group subjected to the delayed injection of lithium chloride did not suppress its saccharin solution intake relative to baseline levels. It is important to note, however, that no water consumption data were reported, and therefore it cannot be deduced whether all fluid consumption was suppressed, or strictly consumption of the saccharin solution.

With all of the evidence, it seems conclusive that GN rats are capable of acquiring taste aversions given proper manipulation of the parameters of the experiment. However, it is not yet obvious whether the GN rats are developing a dislike for the taste as a normal rat would or if they are forming an association between the taste and the subsequent illness. Further examination of the tastant and its role in consumption may clarify the discrepancy.

Taste Reactivity

Taste plays an important role in the decision to ingest or reject food. Ingestion and rejection of tastes have traditionally been measured with fluid consumption tests; two-bottle tests compare ingestion of a tastant with water consumption and one-bottle tests measure mean consumption of a fluid. In both cases, a

measure of palatability is derived from ingestion or rejection. However, rejection does not conclusively indicate disgust or dislike for the substance. Rather, lack of consumption could be due to postingestional satiety. To assess properly the basis of rejection, it is important to distinguish between aversion and avoidance. Stimuli that are innately noxious are considered aversive. Stimuli that are avoided, on the other hand, are treated as such because of several factors (e.g., illness or postingestional satiety).

Intake tests have been the principal method used to measure the role of taste in food consumption or avoidance; the result of these tests is a measure of palatability of the tastant. The taste reactivity test, developed by Grill and Norgren (1978b), allows for evaluation of palatability through analysis of taste-elicited ingestive and aversive responses produced by intraoral infusion of a tastant. Rats will either ingest or reject taste stimuli delivered in this manner; specific patterns of oral behaviors, as well as number and duration of these patterns, provide researchers with a sensitive measure of reactivity.

Grill and Norgren (1978a, 1978b) explain that sucrose and quinine elicit prototypical ingestive and aversive responses, respectively. Additionally, they noted that, after a single pairing of a normally ingestive taste

stimulus with an intraperitoneal lithium chloride injection, the taste stimulus would evoke an exact replica of a quinine response (Grill & Norgren, 1978a). Pfaffmann, Grill and Norgren (1977) reported that when intact rats learned a conditioned taste aversion to normally highly sapid sucrose, their behavioral pattern of rejection imitated the observed natural pattern of rejection to unpalatable quinine solutions.

The difficulty GN rats show in forming taste aversions leads one to question the deficits in the learning or display of the associative process. As cited above, intact rats will substitute rejection for ingestion, usually after just one taste-drug pairing. However, research has not confirmed this to be the case in GN rats as well; it is not known just how GN rats will react to the conditioned tastant. Taste reactivity tests could aid in the delineation of what GN rats learn with repeated trials of taste-illness pairing.

Taste reactivity tests evolved because of the need to distinguish between ingestive and aversive responses to an experimental fluid. In a taste reactivity test, the subject must actively respond to the stimulus for the experimenter. Measures can then be taken to determine if the rat actually likes or dislikes the fluid, as opposed to a one- or two-bottle test, in which the experimenter can only measure consumption, thus assuming

that an ingested amount was enjoyed, and the remainder was left either because of satiety or aversion.

Goltz (1892) and Miller and Sherrington (1915) noted patterns of ingestion and rejection in dogs with ablated cerebral hemispheres and acute decerebrate cats, respectively (Grill & Norgren, 1978c). Grill and Norgren opened the field to taste reactivity tests in rats when they began to study rats that would no longer nourish themselves because of chronic decerebration. They realized that a precise method of measurement of the dependent variable was necessary in extreme cases of brain damage; the taste reactivity test satisfied this requirement by enabling quantification of the sequencing, timing, and morphology of ingestion and rejection responses (Grill & Norgren, 1978c). Their preliminary studies led to the definitions of the various mouth and body responses involved in identifying consummatory behaviors as either ingestive or aversive. For a complete detailed definition of these responses, refer to Grill and Norgren (1978b, 1978c). In brief, these behaviors can be described as follows: "Mouth movements" are bilateral, low amplitude rhythmic movements of the jaw, similar to chewing in a human. "Tongue protrusions" are of the same nature as mouth movements, but the tongue can be seen to extend beyond the plane of the upper incisors. A more familiar, but

somewhat exaggerated example would be a cat lapping milk. "Lateral tongue protrusions" are unilateral with the tongue emerging on one side of the mouth, extending the upper lip and separating the septum.

"Gaping", the prototypical aversive response, begins with the mandible retracting and the corners of the mouth pulling back dorsally to form a triangle. During gapes, the lower lip retracts and the lower incisors project somewhat forward and apart. Gapes occur rapidly, and often in clusters, although this is not required for qualification. Gapes do not necessarily involve loss of fluid. "Passive dripping" is a common aversive response; the rat simply allows the fluid to drip from its mouth, making no attempt to ingest it. "Chin rubbing" involves the animal bowing or dipping its head to the floor and rubbing it along the ground in a forward motion while expelling fluid. "Paw pushing" consists of the animal rubbing its forepaws independently of each other in a back and forth manner on the floor or wall of the chamber. "Head shake" involves rapid side to side, "wet dog" type shaking. It is usually accompanied by "fluid expulsion" (accumulated fluid in the mouth is ejected), thus dispelling the fluid from the oral cavity. "Forelimb flail" is similar to head shaking, but only the forelimbs are involved. Forelimb flail is often preceded by "paw licking"

(licking or spitting on the paws) so as to expel the undesirable fluid. Paw licking usually occurs in bouts, as opposed to discrete trials.

"Face washing" entails a typical grooming behavior of the rat where it cups its paws together while in a sitting position, licks them and then brings them over its head, behind its ears and down the bridge of its face, and ending over its nose. "Locomotion" involves the animal walking around in the test chamber. It is suggested that there is a negative correlation between amount of locomotion and palatability of the tastant; as the palatability decreases, the rat tries to "get away" from the taste by locomoting.

Aversion or Avoidance

Experiments to date have proven conclusively that rats lacking GN can learn to avoid certain tastants under certain conditions. The true nature of the avoidance, however, has never been studied; it has been assumed that, once GN rats form taste aversions, these aversions are the same as aversions in normal rats.

Purpose

The present experiment was designed to determine whether the acquired taste aversion in the GN rat is a true taste aversion or a simple taste avoidance. By using taste reactivity tests on GN rats trained to avoid a taste stimulus, the resultant patterns of responding

should lead to a conclusion about the basis of the acquired taste aversion. Aversive responses would suggest that the GN rats were developing an aversion to the tastant as a normal, intact rat would. Ingestive responses would suggest that the GN rats were simply learning to avoid the solution because of prior pairing with drug-induced illness, but they were not developing an aversion to the tastant.

Experiment 1

Method

Subjects. Twenty-five male Holtzman-derived rats (Sasco, Inc.) 55-60 days of age upon arrival were used. Rats were housed individually with food and water available ad libitum in a room on a 12 hour light/dark cycle with the light cycle beginning at 0700.

Surgery and Histology. Twenty-four hours prior to surgery, rats were food and water deprived. Rats were anesthetized with sodium pentobarbital (55 mg/kg ip) and placed in a nontraumatic headholder. Following skin incision and retraction of the muscles, small holes were made in the lateral aspect of the skull with a drill. The holes were then enlarged with rongeurs and the GN aspirated with a fine-tipped glass pipette. The middle cerebral artery and rhinal sulcus served as landmarks to guide the aspiration. Control ablations involved

somatosensory tissue just dorsal to the GN area of the brain.

Concurrent with cortical ablations, rats were implanted unilaterally with an intraoral cannula. A small piece of polyethylene tubing was inserted anterolateral to the first molar and passed through the cheek muscle, caudal to the eye, up through the incision in the skull. The incision was sutured and a teflon washer was placed over the polyethylene tubing and positioned flush against the skin. A metal fistula was inserted into the polyethylene tubing and the junction was sealed with dental acrylic. Normal control rats were anesthetized and implanted with an intraoral fistula using the same procedure as described. During postoperative recovery, rats were given wet mash until they began eating solid pellets. Operated rats were given a minimum of 3 weeks postoperative recovery.

Following the termination of experimental testing, all rats were given lethal doses of sodium pentobarbital. Lesioned rats were then perfused intracardially with 9% saline solution followed by 10% formol saline. The brains were removed and stored in formol saline solution. Size and location of the ablations were assessed by visual examination; surface drawings of the ablations were made on standard diagrams.

Aversion training and testing. Following postoperative recovery, rats were placed on a schedule of restricted fluid access where solutions were available for 10 min in the morning (11:00 a.m.) and again for 15 min in the afternoon (3:00 p.m.). All training and testing occurred during the morning period. Distilled water was always presented during the afternoon period to allow the rats to maintain adequate hydration during the experiment. Fluids were presented in 50 ml calibrated drinking tubes with one-hole rubber stoppers and stainless steel drinking spouts. Amount of fluid consumed during the morning trials was recorded to the nearest 0.5 ml.

Rats were habituated to the restricted fluid access schedule for a period of 10 days. Following the last day of habituation, the control rats and GN rats were separated into two groups based on mean water consumption. On the eleventh day, sucrose aversion training began. One GN group (trained GN, n=6) and one control group (trained control, n=7; 3 normal control, 4 control lesion) were trained to avoid a .1 M sucrose solution. Immediately following presentation of the sucrose solution, rats were intubated with lithium chloride (3% body weight of a .15 M solution). The remaining GN rats (untrained GN, n=6) and control rats (untrained control, n=6; 3 normal control and 3 control

lesion) were intubated with equimolar sodium chloride solution equivalent in volume to the lithium chloride treatment following sucrose presentation.

Acquisition trials were done every third day with water days in between. Conditioning was discontinued for a trained rat when it consumed less than 0.5 ml of sucrose on an acquisition day. A maximum of five training trials were given. The untrained rats were yoked with the trained rats. When a trained rat achieved criterion, it and its yoked control were tested for taste reactivity.

Taste reactivity testing. Rats were tested for taste reactivity in a clear plastic chamber with a removable lid through which an infusion tube passed. The liquid was infused using a Sage Instruments Model 351 infusion pump at a flow rate of 1 ml/min. A delivery tube was attached to the intraoral cannula for direct infusion of test liquids into the oral cavity. A mirror was positioned under the test chamber to reflect the image of the floor of the test chamber. A Panasonic videocamera with a tele-extender lens positioned approximately 3 feet from the mirror allowed for close up video taping of facial responses for subsequent frame-by-frame analysis.

Rats were habituated to the test chamber during the last two days of the restricted water access period and

every day following until they were tested. Habituation took place after the morning water access period. On the day that rats reached aversion criterion, taste reactivity tests were done. Rats were first given a taste reactivity test using distilled water to serve as a baseline for responses, followed by a 5 min break. Next, they were tested for sucrose taste reactivity. Following the sucrose response testing, rats were given another 5 min break and then tested with .0005 M quinine hydrochloride.

For an individual trial, a rat was placed in the test chamber with the infusion tubing attached to the cannula and allowed to orient itself for approximately 1 min. The video camera and infusion pump were turned on simultaneously and the rat was filmed for 60 sec beyond the first response. Subsequent frame-by-frame film analysis yielded 600 data "scores", one response per tenth-second. Ingestive scores were mouth movements, tongue protrusions, and lateral tongue protrusions (as defined by Grill and Norgren, 1978a). Responses considered as aversive or negative were gaping, passive dripping, head shaking, fluid expulsion, forelimb flail, chin rubbing and paw pushing. There was also a neutral category which signified neither ingestive nor aversive responses. Included in this category were face washing, grooming, paw licking, and locomotion.

Additional training and testing of control groups. Following taste reactivity testing, rats in the trained control group (n=6) were adapted to the restricted fluid access schedule in drinkometer boxes. Rats were adapted to the schedule using distilled water for 3 days. On the fourth day, rats were given sucrose in the drinkometer boxes as a normal acquisition trial (i.e. intubated with lithium chloride). Rats were then returned to normal restricted access fluid schedule (in the home cage) for an additional three days. On the following day, rats were tested for taste reactivity to sucrose without having had the morning period of fluid access.

Additional experimental manipulations were given to the untrained control group (n=6) following sucrose testing. This group of rats was trained to avoid .15 M sodium chloride in the same manner as the sucrose avoidance training. Rats were presented with sodium chloride every third day and intubated with lithium chloride until they reached criterion (drank less than 0.5 ml). On the day a rat reached criterion, it was tested for taste reactivity to sodium chloride (deprived state). The day after taste reactivity testing, rats were given a normal morning water trial before being retested for taste reactivity to sodium chloride (sated state).

Data Analysis. Taste reactivity responses to water, sucrose, and quinine were videotaped and the resulting trials were analyzed frame-by-frame by advancing the videotape and simultaneously recording each response on a scoring sheet. These scoring sheets divided the 60 sec trial into 600 blocks; each block was filled with the response which occurred at that .1 second in the trial. When the animal was not making a response, "no response" was recorded. When the animal's mouth was not visible, "no data" was recorded. The data from the scoring sheets were transferred into microcomputer files and summed. The sums were then transferred into a mainframe computer for statistical analysis.

Specific responses were treated both separately and grouped together for analysis. Mouth movements, tongue protrusions, and lateral tongue protrusions were analyzed as ingestive. Gapes, passive dripping, head shakes, fluid expulsion, forelimb flailing, chin rubbing and paw pushing were analyzed as aversive. Face washing, paw licking, grooming, and locomotion were analyzed individually as neutral responses.

Statistical Treatment. A 2x2 ANOVA (lesion x training) was used to determine if there were significant differences in taste reactivity between normal rats and rats with GN lesions after both groups were trained to avoid sucrose. A separate ANOVA was run

for ingestive and aversive responses, with subsequent analyses of individual responses.

Results and Discussion

Histology. The surface diagrams portraying the GN and control lesions for the present study are presented in Figure 1. The GN lesions were centered on the area defined by Benjamin and Akert (1959) as the "taste nerve area": The lesions extended ventrally to the rhinal sulcus and in some instances encroached upon the piriform cortex. The anterior-posterior range varied moderately. The control lesions centered on the somatosensory neocortex without extending into the gustatory region.

Aversion acquisition. Results from Experiment 1 indicated that both groups of trained rats lowered sucrose consumption over trials (see Figure 2). Mean consumption on the first acquisition trial showed no significant differences between the GN and control groups. On the second acquisition trial, significant differences were found between GN and control rats, $F(1,21) = 16.71$, $p < .001$, and between trained and untrained groups, $F(1,21) = 28.69$, $p < .001$. Additionally, a significant interaction was found, F

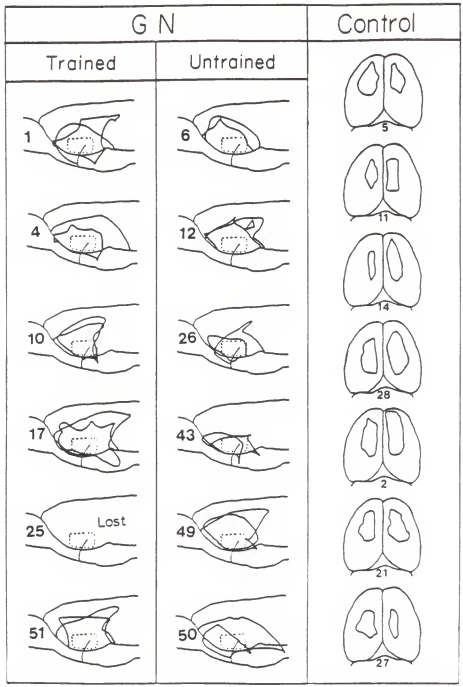

Figure 1. Histological surface diagrams from Experiment 1. The dashed line indicates the "taste nerve area" as defined by Benjamin & Akert (1959). Trained control rat brains are: 5, 11, 14, & 28. Untrained control rat brains are: 2, 21, & 27.

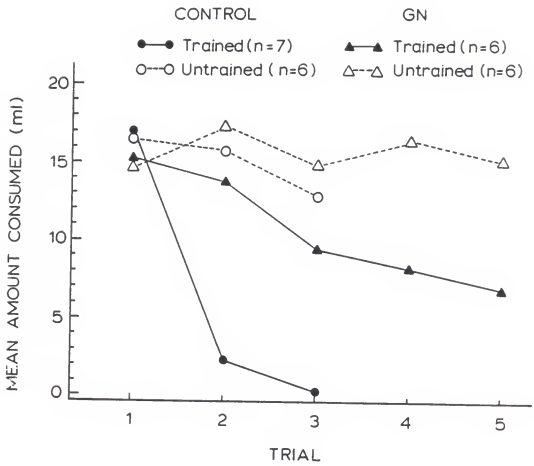

Figure 2. Mean amount of sucrose consumed during acquisition trials in Experiment 1.

(1,21) = 10.17, p = .004. Post hoc comparisons revealed that the trained control group differed significantly from the trained GN group and from the untrained control group, F (1,21) = 11.63, p < .01; F (1,21) = 16.20, p < .001. Two trained rats reached criterion and they and one yoked control rat were excluded from further training and were not included in the analysis of trial three. Similar results were found on the third acquisition trial, where only the trained control group consumed significantly less sucrose than the other three groups (which did not differ significantly). Significant differences for the third acquisition trial were found between GN and control groups, F (1,18) = 10.99, p = .004, and between trained and untrained groups, F (1,18) = 29.23, p < .001. A significant interaction was also found, F (1,18) = 4.65, p < .05, and post hoc analysis showed the trained control group to differ significantly from both the trained GN group and the untrained control group, F (1,21) = 9.22, p <.01; F (1,21) = 12.09, p < .01. On acquisition day 4, the trained GN group drank less than the untrained GN group, t (10) = 4.56, p < .01. On acquisition day 5, one trained GN rat reached criterion. As on acquisition day 4, untrained GN rats drank significantly more than trained GN rats, t (10) = 4.42, p < .01.

Taste reactivity responses. Figure 3 shows the number of rats that made aversive responses to the three different solutions. Two of the trained control rats were tested for taste reactivity after one lithium chloride intubation, and the other five were tested after two intubations. One trained GN rat was tested after four pairings of sucrose and lithium chloride; the remaining five trained GN rats were tested after five intubations. As can be seen in Figure 3, four out of seven trained control rats gaped to sucrose, compared to zero trained GN rats.

Aversive responding to quinine was high for both control and GN rats. All rats gaped, and more control rats did head shaking, forelimb flailing, and fluid expulsion than did GN rats. Trained control and trained GN rats actively chin rubbed; their untrained counterparts were not as active, but did chin rub to quinine more so than with any other tastant. Two trained control rats and one trained GN rat gaped to water. Nine out of twelve GN rats (4 trained, 5 untrained) passively dripped water, while only 3 out of thirteen control rats did. Half (n=3) of the trained GN rats head shook to water, and half (n=3) of the untrained GN rats did forelimb flailing. This was accompanied by fluid expulsions in only one untrained GN rat.

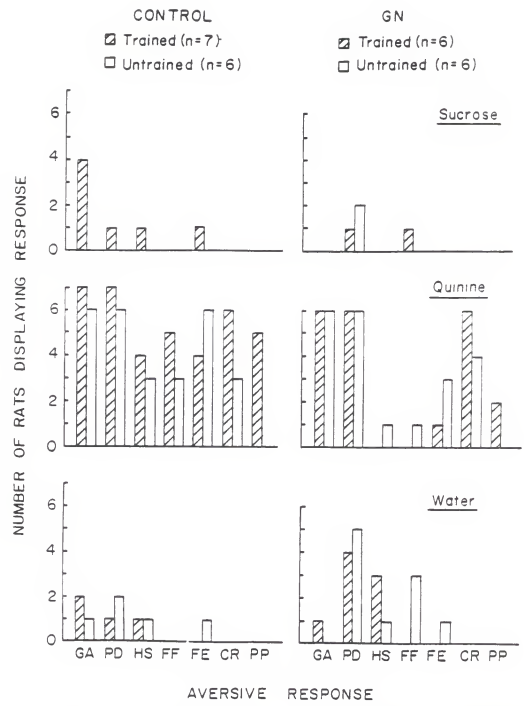

Figure 3. Number of rats making aversive responses to sucrose, quinine, and water in Experiment 1. (Abbreviations: GA=gape; PD=passive drip; HS=head shake; FF=forelimb flail; FE=fluid expulsion; CR=chin rub; PP=paw push)

Table 1 depicts mean number of individual responses to sucrose. All rats made ingestive responses to sucrose with the exception of three rats not making any lateral tongue protrusions. Control rats made significantly more mouth movements than GN rats, $F(1,21) = 28.05$, $p < .001$. No significant differences were found for total ingestive responses: Mean number of total ingestive responses (mouth movements, tongue protrusions, and lateral tongue protrusions) was: trained control = 243.57 ($\pm$ 15.20); untrained control = 227.17 ($\pm$ 29.69); trained GN = 178.33 ($\pm$ 12.67); untrained GN = 175.00 ($\pm$ 16.43). Total aversive responses (gapes, passive drips, head shakes, forelimb flails, fluid expulsion, chin rubs, and paw pushes) were slightly higher for the trained control group, but no statistically significant effects were found for aversive responses overall (see also Figure 3).

Table 2 shows the mean number of individual reactivity responses to quinine (refer also to Figure 3). There were fewer ingestive responses to quinine, although all rats did make mouth movements and tongue protrusions; only five rats made lateral tongue protrusions. Mean number of total ingestive responses was: trained control = 75.14 ($\pm$ 13.85); untrained control = 38.67 ($\pm$ 9.53); trained GN = 52.00 ($\pm$ 13.02); untrained GN = 74.17 ($\pm$ 22.98). Trained rats made

Table 1.

Mean number (±SEM) of reactivity responses to sucrose in Experiment 1.

		INGESTIVE			AVERSIVE					
CONDITION	MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
TRAINED										
CONTROL n=7	80.71 ±12.47	155.14 ±9.90	7.71 ±2.12	3.14 ±1.90	0.14 ±.14	0.14 ±.14	0.00 ±.00	0.14 ±.14	0.00 ±.00	0.00 ±.00
GN n=6	23.00 ±7.36	147.17 ±13.33	8.17 ±1.89	0.00 ±.00	0.67 ±.67	0.00 ±.00	0.17 ±.17	0.00 ±.00	0.00 ±.00	0.00 ±.00
UNTRAINED										
CONTROL n=6	82.83 ±13.30	139.17 ±38.72	5.17 ±2.85	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
GN n=6	26.67 ±6.40	142.17 ±18.87	6.17 ±2.88	0.00 ±.00	0.50 ±.34	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00

Table 2.

Mean number (±SEM) of reactivity responses to quinine in Experiment 1.

		INGESTIVE			AVERSIVE					
CONDITION		MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
	CONTROL n=7	35.71 ±11.61	39.14 ±5.98	0.29 ±0.18	29.17 ±3.21	9.14 ±1.53	1.57 ±.61	1.57 ±.57	2.14 ±.80	2.71 ±.81	6.43 ±2.50
TRAINED											
	GN n=6	4.00 ±1.46	47.33 ±11.82	0.67 ±.49	30.50 ±3.13	12.17 ±2.46	0.00 ±.00	0.00 ±.00	0.17 ±.17	5.83 ±1.01	3.50 ±2.78
	CONTROL n=6	12.33 ±3.02	26.33 ±7.34	0.00 ±.00	15.83 ±3.36	11.17 ±2.30	1.83 ±1.14	2.67 ±2.12	3.50 ±.96	3.17 ±1.68	0.00 ±.00
UNTRAINED											
	GN n=6	20.83 ±12.28	52.67 ±12.17	0.67 ±.67	25.33 ±4.08	16.50 ±1.52	0.17 ±.17	0.17 ±.17	0.67 ±.33	1.33 ±.61	0.00 ±.00

significantly more total aversive responses than did their untrained counterparts, F (1,21) = 7.94, p = .01. A look at individual negative reactivity responses shows that trained rats made significantly more gapes than untrained rats, F (1,21) = 7.57, p = .01, but GN rats made more passive drips than control rats, F (1,21) = 4.49, $p < .05$.

Table 3 presents a breakdown of individual responses to water. All rats made ingestive responses to water; nine rats did not make any lateral tongue protrusions. Control rats made significantly more mouth movements than GN rats, F (1,21) = 9.53, p = .01. Control rats also made more tongue protrusions than GN rats, but not significantly more. GN rats had a larger number of passive drips than control rats. However, this was also found with the quinine solution; GN rats passive dripped more than control rats.

Additional training and testing. Results from the additional sucrose test indicated that five of the six rats retained their aversions in the drinkometer boxes (i.e., consumed 0.5 ml or less); mean consumption was 0.58 ml ($\pm$ 0.20). The sixth rat drank 1.5 ml. The taste reactivity data from all six rats taken after the sucrose-illness trial in the drinkometer boxes can be seen in Table 4. All rats gaped and most rats passive dripped. All rats made at least some ingestive

Table 3.

Mean number (±SEM) of reactivity responses to water in Experiment 1.

		INGESTIVE			AVERSIVE					
CONDITION		MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
TRAINED	CONTROL n=7	32.43 ±7.82	152.57 ±22.24	7.43 ±2.37	1.29 ±.89	0.14 ±.14	0.14 ±.14	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
	GN n=6	12.17 ±3.47	102.33 ±43.84	3.17 ±1.47	0.50 ±.50	7.33 ±3.04	0.50 ±.22	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
UNTRAINED	CONTROL n=6	44.50 ±9.93	114.00 ±32.29	4.00 ±2.86	0.17 ±.17	0.67 ±.49	0.33 ±.33	0.00 ±.00	0.50 ±.50	0.00 ±.00	0.00 ±.00
	GN n=6	18.17 ±7.21	65.67 ±16.72	3.17 ±1.68	0.00 ±.00	8.00 ±3.42	0.33 ±.33	0.67 ±.33	0.17 ±.17	0.00 ±.00	0.00 ±.00

Table 4.

Mean number (±SEM) of reactivity responses to sucrose for rats trained in drinkometer boxes in Experiment 1.

	INGESTIVE			AVERSIVE						
CONDITION	MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
TRAINED CONTROL	20.67 ±8.31	106.00 ±31.84	3.00 ±1.61	7.33 ±2.01	6.50 ±3.32	1.17 ±.48	0.00 ±.00	0.50 ±.22	0.00 ±.00	0.00 ±.00

responses; four rats made mouth movements, all six rats made tongue protrusions, and three rats made lateral tongue protrusions.

In the additional sodium chloride test, all rats reached criterion after only one (n=3) or two (n=3) pairings of sodium chloride and illness. The results from the two taste reactivity tests, one during a period of fluid depletion and the other after the rats were relatively fluid replete, are listed in Table 5. As can be seen, ingestive responses decreased and aversive responses (especially passive drips) increased when the animals were sated as opposed to being in the deprived or hungry state. The mean number of aversive responses in the hungry and sated states were 19.67 ($\pm$ 8.98) and 38.67 ($\pm$ 3.38), respectively. In general, those rats that received two pairings of lithium chloride made more aversive responses and less ingestive responses than those rats that received only one pairing of sodium chloride and illness.

Analysis of the results from Experiment 1 indicated that acquisition of the aversion was rapid for trained control rats (i.e. in one or two trials of sucrose-lithium chloride pairing). Although trained control rats learned an aversion to sucrose, negative taste reactivity was found to be relatively weak.

Table 5.

Mean number (±SEM) of reactivity responses to sodium chloride for control rats trained to avoid sodium chloride in Experiment 1.

	INGESTIVE			AVERSIVE						
CONDITION	MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
WATER DEPRIVED	33.00 ±5.20	129.67 ±26.32	3.67 ±1.65	1.83 ±.54	2.50 ±1.45	1.50 ±.85	2.00 ±1.81	1.83 ±1.33	0.50 ±.34	0.00 ±.00
WATER SATED	13.50 ±5.34	38.67 ±13.88	0.17 ±.17	0.67 ±.67	10.67 ±2.29	1.50 ±.76	3.83 ±1.08	2.67 ±.56	0.00 ±.00	0.00 ±.00

Results from the sucrose study on the control rats in the drinkometer boxes showed the sucrose aversions were not situationally specific. This test eliminated any notion that a place avoidance was present because, upon first presentation of sucrose in the drinkometer boxes, five of the six rats maintained criterion. Negative reactivity was not strong after the initial acquisition trials and increased slightly after an additional training trial in the drinkometer boxes. Therefore, perhaps due to its caloric value and it being highly nutritive, sucrose may not be an optimal conditional stimulus for deprived rats in a taste aversion learning paradigm. Because the rats were hungry when being tested for taste reactivity, the nutritive qualities of sucrose may have overridden the aversive properties.

GN rats, on the other hand, made little or no aversive responding, and it would appear that they did not learn an aversion. Only one trained GN rat reached criterion; all other trained GN rats lowered their sucrose consumption over training trials, and thus appeared to be "approaching" criterion, but more sucrose-lithium chloride pairings would have been necessary. Even then, it is not certain if the trained GN rats would ever have reached criterion.

Experiment 2

Experiment 2 was designed to test three important components found in Experiment 1. First, it was found in Experiment 1 that, even though some trained control animals learned an aversion and displayed negative reactivity responses after one tastant-drug pairing, those rats that received two lithium chloride intubations displayed stronger aversions (i.e., more negative reactivity). Second, it was shown that sucrose was not an optimal conditional stimulus for taste reactivity tests in deprived rats but that sodium chloride was the solution of choice. Third, it was demonstrated that rats allowed to be fluid replete prior to reactivity testing showed stronger, more negative reactivity to the conditional stimulus.

With the above factors in mind, Experiment 2 was designed to intensify the negative reactivity found in rats trained to avoid a specific taste. Sodium chloride was substituted for sucrose as the conditional stimulus. Control rats were given three tastant-drug pairings, and GN rats were given four. All rats were tested for taste reactivity after a 10 min water access period (sated state). Additionally, acquisition trials were spaced every 48 hours, as opposed to every 72 hours as in Experiment 1.

Method

Subjects. Thirty-two naive, male Holtzman-derived rats (Sasco, Inc.) 55-60 days of age upon arrival were used. Rats were housed individually with food and water available ad libitum in a room on a 12 hour light/dark cycle.

Surgery and Histology. Rats were anesthetized and cortical lesions and fistulae implants were performed the same as in Experiment 1. Rats were allowed wet mash for as long as needed to insure adequate weight gain. Operated rats were given 2 weeks postoperative recovery.

Following termination of experimental testing, all rats were given lethal doses of sodium pentobarbital. Rats with lesions were then perfused and their brains were removed and analyzed in the same manner as in Experiment 1.

Aversion training and testing. Following postoperative recovery, rats were placed on a restricted fluid access schedule identical to that in the first experiment. After 10 days habituation to the schedule, sodium chloride aversion training began. Both GN and control rats were divided into subgroups based on mean water consumption. One GN group (n=8) and one control group (n=8; 4 normal control, 4 control lesion) were trained to avoid .15 M sodium chloride by pairing its presentation with immediate intubations of lithium

chloride (3% body weight of a .15 M solution). The remaining GN rats (n=8) and control rats (n=8; 4 normal control, 4 control lesion) were intubated with an equimolar sodium chloride solution equivalent in volume to the lithium chloride treatment.

Training sessions were done every other day with water days in between. After three acquisition trials, control rats were tested for taste reactivity. GN rats were tested after four acquisition trials.

Taste reactivity testing. Rats were tested for taste reactivity in the same chamber and using the same instruments used in Experiment 1. Rats were habituated to the test chamber on the last day of the restricted water access period and every day following until they were tested. Rats received the morning water trial before each testing. Rats were first given a taste reactivity test using distilled water to serve as a baseline for responses. Following a 5 min rest, rats were tested for sodium chloride reactivity. Rats were then returned to the home cage and maintained on the restricted fluid access schedule. The following morning, rats were again presented with sodium chloride to confirm that criterion had been reached.

Data analysis. Taste reactivity responses to the water and sodium chloride solution were videotaped and

the resulting trials analyzed in the same manner as in Experiment 1.

Statistical Treatment. A 2x2 ANOVA (lesion x training) was used to determine if there were significant differences in taste reactivity between normal rats and rats with GN lesions after both had been trained to avoid sodium chloride. A separate ANOVA was run for ingestive, aversive, and neutral categories of responses, with subsequent analyses of individual responses.

Results and Discussion

Histology. Figure 4 presents surface diagrams of the GN and control lesions for Experiment 2. In general, lesions were similar to those in Experiment 1; the lesions were found to center on the "taste nerve area" as defined by Benjamin and Akert (1959). The control lesions centered on the dorsal aspect of the somatosensory neocortex without encroaching upon the gustatory region.

Aversion acquisition. Results from Experiment 2 revealed a decline in sodium chloride consumption for trained rats over trials (see Figure 5). No significant differences between groups for mean sodium chloride consumption were found on the first acquisition day. On the second acquisition day, significant differences were found between control and GN groups, F (1,28) = 6.04,

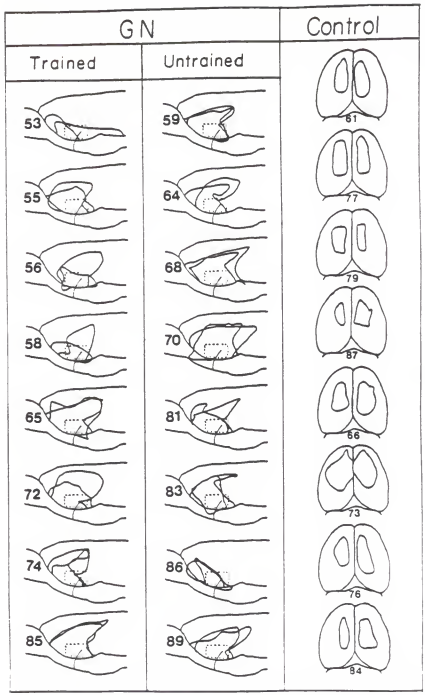

Figure 4. Histological surface diagrams from Experiment 2. The dashed line indicates the "taste nerve area" as defined by Benjamin & Akert (1959). Trained control rat brains are: 61, 77, 79, & 87. Untrained control rat brains are: 66, 73, 76, & 84.

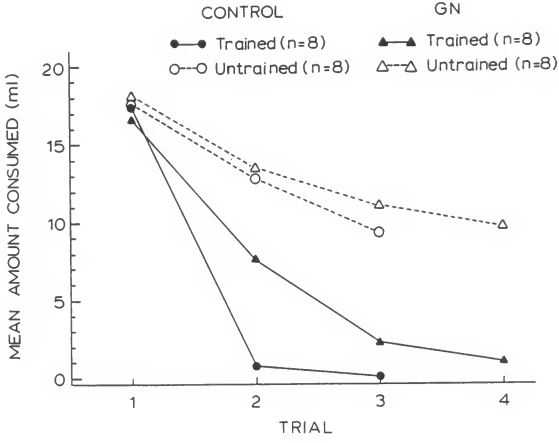

Figure 5. Mean amount of sodium chloride consumed during acquisition trials in Experiment 2.

$p = .02$, with control rats drinking less, and between trained and untrained rats, $F\ (1,28) = 33.81$, $p < .001$, with trained rats drinking less. A significant interaction was found, $F\ (1,28) = 4.03$, $p = .05$, and post hoc comparisons indicated a significant difference between the trained control and the trained GN group, $F\ (1,28) = 4.98$, $p < .05$, and between the trained control and the untrained control group, $F\ (1,28) = 15.30$, $p < .001$, with the trained control group consuming less sodium chloride solution in both instances. On the third acquisition day, a significant difference was found between trained and untrained rats $F\ (1,28) = 60.74$, $p < .001$. Mean consumption was 1.06 ml for trained rats and 10.12 ml for untrained rats. All control rats got three intubations, and all GN rats got four intubations. All control rats reached criterion by the third trial, and six of the eight GN rats achieved criterion by the fourth trial. Of the remaining two GN rats, one drank 1.5 ml and the other drank 6.5 ml on the fourth acquisition day.

Taste reactivity responses. Figure 6 details the number of rats making aversive responses. More than half of the trained control rats gaped to sodium chloride compared to zero rats in any other group. More trained control rats displayed the responses of head shake, forelimb flail, fluid expulsion, and chin rubbing

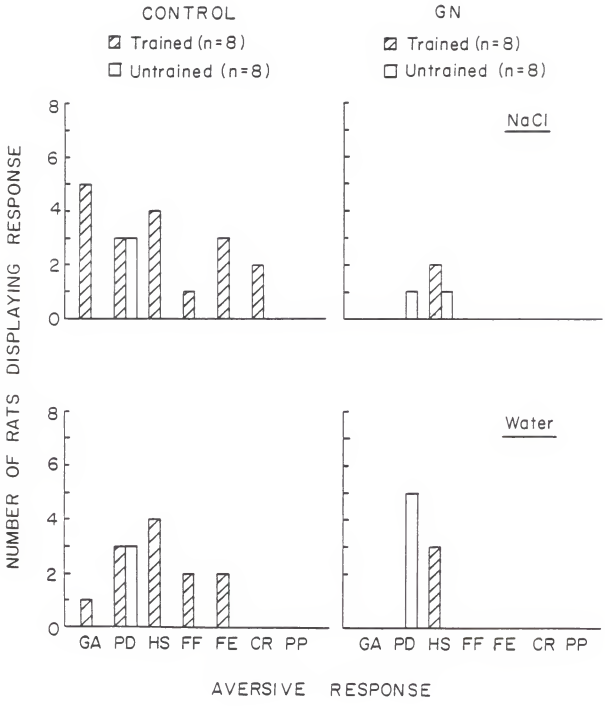

Figure 6. Number of rats making aversive responses to sodium chloride and water in Experiment 2. Abbreviations: see Figure 3.

than any other group. GN rats made relatively few aversive responses to sodium chloride overall. Trained control rats made more aversive responses to water than any other group, with the exception of untrained GN rats making more passive drips. All rats made ingestive responses to both solutions. Ten rats failed to make any lateral tongue protrusions to sodium chloride, and three rats made no mouth movements to sodium chloride. Fifteen rats did not make any lateral tongue protrusions to water.

Table 6 shows mean number of individual reactivity responses to sodium chloride. Significant differences for total aversive responses were found for lesion, F (1,28) = 17.90, $p < .001$, and for training, F (1,28) = 6.82, $p = .01$. Control rats made more aversive responses to sodium chloride than did GN rats, and trained rats made more aversive responses than did untrained rats. A significant interaction, F (1,28) = 6.82, $p = .01$, led to post hoc analysis which showed a significant difference between the trained control group and trained GN group, F (1,28) = 11.71, $p < .01$, as well as a significant difference between the trained and untrained control groups, F (1,28) = 6.83, $p < .025$. As can be seen in Figure 4 as well, the trained control group made significantly more gapes than any other group, F (1,28) = 6.91, $p = .01$. GN rats made

Table 6.

Mean number (±SEM) of reactivity responses to sodium chloride in Experiment 2.

CONDITION	INGESTIVE			AVERSIVE						
	MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
TRAINED										
CONTROL n=8	35.13 ±10.21	76.38 ±22.56	1.63 ±.98	4.50 ±1.71	5.38 ±2.69	0.88 ±.35	0.38 ±.38	0.63 ±.38	0.25 ±.16	0.00 ±.00
GN n=8	20.38 ±8.77	297.13 ±22.57	6.38 ±1.18	0.00 ±.00	0.00 ±.00	0.38 ±.26	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
UNTRAINED										
CONTROL n=8	27.00 ±5.69	111.00 ±23.21	0.38 ±.18	0.00 ±.00	3.13 ±1.99	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
GN n=8	28.00 ±8.38	186.25 ±32.86	5.38 ±1.61	0.00 ±.00	0.13 ±.13	0.25 ±.25	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00

significantly more ingestive responses than did the control rats, F (1,28) = 33.69, $p < .001$. There was also a significant interaction, F (1,28) = 6.27, p = .01; post hoc analysis showed trained GN rats made more ingestive responses than untrained GN rats, F (1,28) = 4.29, $p < .05$, and also trained GN rats made more ingestive responses than trained control rats, F (1,28) = 17.55, $p < .001$. For the response of tongue protrusions, the GN group made significantly more than the control group, F (1,28) = 33.22, $p < .001$. A significant interaction between lesions and training was found, F (1,28) = 8.03, $p < .01$; post hoc comparison showed the trained GN group made more tongue protrusions than the untrained GN group, F (1,28) = 4.66, $p < .05$, and also more than the trained control group, F (1,28) = 18.48, $p < .001$. Lastly, GN rats differed significantly from control rats on lateral tongue protrusions, F (1,28) = 19.05, $p < .001$, with GN rats making more than control rats.

Table 7 presents individual reactivity responses to water. No significant differences were found between groups for total aversive responses (see also Figure 4). However, there was a significant difference between the trained and untrained groups when passive dripping was excluded from the total aversive score, F (1,28) = 5.02, $p < .05$, with the trained group making more aversive

Table 7.

Mean number (±SEM) of reactivity responses to water in Experiment 2.

		INGESTIVE			AVERSIVE					
		MM	TP	LT	GA	PD	HS	FF	FE	CR	PP
TRAINED	CONTROL n=8	16.38 ±6.62	88.25 ±30.38	1.13 ±.85	1.25 ±1.25	4.13 ±2.50	0.88 ±.40	0.25 ±.16	0.50 ±.33	0.00 ±.00	0.00 ±.00
	GN n=8	24.13 ±7.97	249.25 ±31.03	7.25 ±1.42	0.00 ±.00	0.00 ±.00	0.50 ±.27	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
UNTRAINED	CONTROL n=8	16.00 ±3.83	103.00 ±26.40	0.25 ±.16	0.00 ±.00	4.75 ±2.50	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00
	GN n=8	26.38 ±5.79	140.13 ±32.17	2.75 ±1.59	0.00 ±.00	4.75 ±2.02	0.13 ±.13	0.00 ±.00	0.00 ±.00	0.00 ±.00	0.00 ±.00

responses. The GN group made more ingestive responses with water than the control group F (1,28) = 14.78, p < .001. There was a significant difference between GN and control rats for tongue protrusions, F (1,28) = 10.85, p = .003, as well as a significant interaction, F (1,28) = 4.24, p = .05. Post hoc comparisons yielded differences between the trained GN rats and trained control rats, F (1,28) 7.16, p < .025, with the trained GN rats making more tongue protrusions. Lastly, GN rats made more lateral tongue protrusions than did control rats, F (1,28) 14.01, p = .001, and trained rats made more than untrained rats, F (1,28) = 5.44, p = .025.

Results from Experiment 2 indicated that the trained control rats rapidly learned an aversion to sodium chloride and displayed strong negative taste reactivity. They were, in fact, the only rats to gape or chin rub to sodium chloride. Trained GN rats also appeared to learn an aversion to the tastant, but negative taste reactivity for these rats was virtually nonexistent. Six of the eight rats in the trained GN group reached criterion on the fourth acquisition trial. However, in contrast to the trained control group, the trained GN group displayed virtually no negative taste reactivity to sodium chloride in the taste reactivity test. The trained GN group was indistinguishable from both the untrained control rats and the untrained GN rats.

GENERAL DISCUSSION

Rats lacking gustatory neocortex (GN) displayed deficits in the acquisition of conditioned taste aversions to both sucrose (Experiment 1) and sodium chloride (Experiment 2), as compared to control rats. Latency to taste aversion acquisition (criterion for taste aversion was consumption of 0.5 ml or less on an acquisition trial) was longer for GN rats than for control rats; 4 or 5 acquisition trials versus 1 or 2 acquisition trials, respectively. Control rats displayed negative reactivity responses to the paired taste when tested for taste reactivity. GN rats, on the other hand, displayed virtually no negative taste reactivity responses.

Previous work has shown that taste aversion learning in a GN rat is seriously impaired compared to a control rat. However, ablation of the GN does not seem to interfere with basic taste detection or acceptance/rejection responses to novel taste stimuli (Braun & Kiefer, 1975; Braun et al., 1982). Deficits seen in taste aversion learning in the GN rat include increased latency to acquisition, weaker display of aversion, quicker extinction, and generalization to unpaired tastes. Evidence from the present study verifies these conclusions. In Experiment 1, rats were

given acquisition trials every 72 hours, which did not result in strong aversions for 5 of the 6 trained GN rats after five trials. However, all trained control rats learned an aversion within two training trials. In Experiment 2, training trials were every 48 hours, and this produced better acquisition results in that six of eight trained GN rats formed aversions after four trials, and all trained control rats developed aversions within two trials.

Aversive responding to sucrose in taste reactivity tests in Experiment 1 was weak for all groups of rats. The trained control group was the only group to gape to the solution; the untrained control group did not display any negative reactivity responses. Trained control rats in Experiment 2 made a higher number of negative responses than the trained control group in Experiment 1. More responses of gape and passive drip were made, as well as head shake, forelimb flail, fluid expulsion and chin rub. The untrained control group in Experiment 2 made some passive drips, but otherwise there was no negative responding.

GN rats displayed little or no negative taste reactivity responses to both sucrose (Experiment 1) and sodium chloride (Experiment 2). One trained and two untrained GN rats in Experiment 1 passive dripped. One trained GN rat made the response of forelimb flail as

well. In Experiment 2, two trained and one untrained GN rats made head shakes, and one untrained GN rat passive dripped. The results clearly indicated that rats lacking GN failed to produce negative reactivity to a taste which had been paired with illness despite the fact that these same rats refused to consume that taste (e.g., Experiment 2).

Despite the low frequency of negative reactivity responses in GN rats, the same rats were capable of producing these responses. Total aversive responding during the quinine taste reactivity tests in Experiment 1 showed GN rats to be indistinguishable from control rats. Such a result was not surprising because Grill and Norgren (1978c) tested decerebrate rats for taste reactivity to quinine and found their response pattern to be identical to that of intact rats.

Results from the present study converge with evidence regarding the role of the GN in learned taste aversions to support the notion that tastes paired with emesis-producing drugs do not produce traditional conditioned taste aversions in the rat lacking GN. It appears that, although GN rats can be trained to avoid a taste stimulus, they do not produce the negative reactivity found in normal control rats. Previous work maintains that the gustatory area of the neocortex plays a significant role in mediating conditioned taste

aversions (Yamamoto et al., 1980): While it may have appeared that GN rats in the present study acquired aversions to the tastants (as evidenced by refusal to consume these tastants), taste reactivity results dispel this. It can only be concluded that GN rats learned to avoid the conditional tastant, but that no hedonic shift in palatability took place as it had in normal rats.

When a novel taste stimulus is paired with an emetic producing drug, a pattern of rejection responses similar to quinine should be elicited during taste reactivity testing (Grill & Norgren, 1978a; Parker, 1982, 1984). However, Parker (1988) found differences in patterns of rejection in taste reactivity tests comparing quinine and sucrose when sucrose had been paired with lithium chloride over four trials. Quinine elicited more gapes and chin rubs than sucrose. Parker suggested that a palatability shift was not necessarily the only mechanism governing avoidance responses because tastants that were equally avoided were not equally rejected. That is, while rats would avoid both quinine and sucrose after it had been paired with lithium chloride, they would more actively reject the quinine solution than the sucrose solution.

Rats in Experiment 1 failed to show much negative reactivity during taste reactivity testing, even though they avoided sucrose during the previous training trial.

Perceived hunger or satiety of the animal is assumed to contribute significantly to taste aversion learning. While it is adaptive behavior to reject a noxious taste stimulus, complete rejection of all nourishment to the point of starvation or dehydration is maladaptive. It is suggested that, because four of the six trained control rats in Experiment 1 gaped to the fluid despite ample consumption during taste reactivity testing, the hunger of the rats motivated them to consume the conditioned stimulus, regardless of prior conditioning. In Experiment 2, rats were allowed to consume water prior to reactivity testing, thus repleting their systems. In comparison, the trained control rats in Experiment 2 showed elevated negative taste reactivity responding compared to trained control rats in sucrose reactivity tests in Experiment 1.

Water also elicited some negative reactivity responses in both experiments. It is not understood why rats display aversive responses to water; factors including palatability and satiety have been explored. It may simply be that water tastes bad; similar negative responses to water have been found in the same laboratory during other taste reactivity tests (unpublished observations). Satiety cannot explain the trained control rats' negative responding to water. These rats had been fluid deprived for 20 hours and had

refused fluid during the morning access period. Additionally, as reported by Grill and Norgren (1978a), response termination by a sated rat (normal or decerebrate) was almost always characterized by a quinine-like pattern of response (gapes and chin rubs). Virtually all of the aversive responses made to water in the present study were passive drips, and these were made in the last few seconds of the 60 sec trial.

Untrained control and untrained GN rats in Experiment 2 decreased sodium chloride consumption across trials. It is possible that the intubation process itself caused a mild aversion. Behavioral observations would support this notion; rats were more agitated on the second and subsequent intubation trials than on the first. It is also possible that the untrained rats experienced gastric distress following intubation; recall that these rats consumed fluid to satiety just prior to being intubated. The intubation amount was roughly the same amount of fluid as had just been consumed, and therefore the rats may have felt uncomfortable gastric distension from being "too full". Results from the present study suggest that normal rats acquire conditioned taste aversions, and GN rats learn simple taste avoidances. Recall that an aversive stimulus is one that is innately noxious, and an avoided stimulus is one that is avoided only after it has been paired with a negative

consequence (i.e. illness). Consequently, an avoided taste stimulus may elicit behaviors similar to an aversive taste stimulus as the result of training. Garcia, Hankins, and Rusiniak (1974) and Grill and Norgren (1978b) have suggested that taste avoidance produced by aversion training (i.e., pairing the taste with lithium chloride) results in a hedonic shift in palatability of the tastant; what was once palatable now is not.

Results from the present experiments tend to confirm a change in the taste of the solution for trained control rats; tastes that were once preferred elicited rejection responses during taste reactivity tests after having been paired with lithium chloride. Data from the trained GN rats do not support a hedonic shift in palatability of the tastant; in both experiments, these rats responded ingestively to sucrose (Experiment 1) and sodium chloride (Experiment 2). Thus, while the trained GN rats may indeed have learned to avoid a tastant, they failed to show a change in palatability of the tastant.

Brain damage often produces specific impairments in an organisms abilities to cope. This does not necessarily mean that the organism is totally incapable of functioning. Rather, brain damaged organisms frequently learn coping mechanisms that allow them to be

partially, if not fully, functional. This appears to be the case with rats lacking gustatory neocortex; GN rats require more acquisition trials to acquire an aversion of the same magnitude as a control rat. GN rats do not tolerate delays well, both between the conditioned and unconditioned stimulus, as well as between training trials. The stimulus used may also play a role in taste aversion learning. As in the present study, three-fourths of the GN rats given sodium chloride paired with lithium chloride every 48 hours for four training trials learned to avoid the solution. Only one GN rat given five sucrose-lithium chloride pairings every 72 hours learned to avoid the tastant.

It is possible that rats lacking GN and normal rats use different strategies to learn taste associations. One of these possibilities is that GN rats rely on postingestional cues to distinguish between tastes. In one experiment, Phillips (1977) exposed groups of normal rats and GN rats to a sodium chloride-lithium chloride discrimination test and found that normal rats had no trouble discriminating between the two solutions over three trials (lithium chloride consumption was suppressed to zero, and sodium chloride consumption approached water baseline level). GN rats, on the other hand, drank roughly 10 ml sodium chloride and 5 ml lithium chloride on each of the trials (cited in Braun

et al., 1982). This suggested that GN rats quit drinking lithium chloride only after they began experiencing postingestional illness, thus alerting them to the fact that the solution they were drinking was lithium chloride and not sodium chloride.

GN rats appear to be using some strategy other than palatability shifts to learn to avoid a taste. One possibility is that the trained group of GN rats used situational cues to aid them in learning to avoid the paired tastant. Variants including time of day (training trials took place during the morning fluid access period only), and behavioral patterns of the experimenter are included. On acquisition days, two experimenters were present instead of one, as opposed to normal water days. Rats were weighed and then food was removed from their cages before acquisition training began (food was not removed from the cages on water days). Sodium chloride bottles were put on the cages one per minute, as opposed to one per 15 seconds with water bottles. Even if the GN rats were hedonically naive, they might have been able to learn to avoid the solution by using the salient environmental cues.

The taste stimuli used in the present experiment may propose an additional explanation. Sucrose and sodium chloride may simply have tasted too good to the trained GN rats. It has been shown that GN rats are

hyperresponsive to sapid stimuli such as sucrose and sodium chloride (Braun et al., 1982). It can be argued that certain sapid stimuli taste better to GN rats than to normal rats, and therefore more conditioning would be required in the GN rat than in the normal rat to result in a conditioned aversion. Whether the GN rat would ultimately display negative reactivity responses remains to be seen.

It remains apparent that GN rats do not experience a shift in palatability to a tastant that has been paired with illness, as normal rats do. This may be used to explain why a GN rat has difficulty in forming an avoidance to a taste. The GN rat must rely solely on prior experience with the tastant to learn to avoid the solution, but the normal rat encounters unpleasant taste cues in addition to having experienced illness on previous trials involving the tastant. Thus, conditioning a GN rat to avoid a tastant by using illness-producing drugs may be no different from using foot shock, for example. Both are punishing, but do not alter the perceived hedonic value of the tastant for GN rats.

Results from the present study clearly show that the gustatory neocortex is paramount in taste association learning; rats lacking GN do not form conditioned taste aversions. GN rats can learn to avoid a tastant though.

Thus, learning impairments in GN rats may be exclusive to taste-illness associations, a hypothesis which needs to be explored in further research.

REFERENCES

Benjamin, R.M., & Akert, K. (1959). Cortical and thalamic areas involved in taste discrimination in the albino rat. Journal of Comparitive Neurology, 111, 231-260.

Benjamin, R.M., & Pfaffman, C. (1955). Cortical localization of taste in albino rat. Journal of Neurophysiology, 18, 56-64.

Braun, J.J., & Kiefer, S.W. (1975). Preference-aversion functions for the basic taste stimuli in rats lacking gustatory neocortex. Bulletin of the Psychonomic Society, 6, 438-439 (Abstract).

Braun, J.J., Kiefer, S.W. & Ouellet, J.V. (1981). Psychic ageusia in rats lacking gustatory neocortex. Experimental Neurology, 72, 711-716.

Braun, J.J., Lasiter, P.S., & Kiefer, S.W. (1982). The gustatory neocortex of the rat. Physiological Psychology, 10, 13-45.

Braun, J.J., Slick, T.B, & Lorden, J.F. (1972). Involvement of gustatory neocortex in the learning of taste aversions. Physiology and Behavior, 9, 637-641.

Garcia, J., Hankins, W., & Rusiniak, K. (1974). Behavioral regulation of the milieu interne in man and rat. Science, 185, 824-833.

Grill, H.J., & Norgren, R. (1978a). Chronically decerebrate rats demonstrate satiation but not bait shyness. Science, 201, 267-269.

Grill, H.J., & Norgren, R. (1978b). The taste reactivity test I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263-279.

Grill, H.J., & Norgren, R. (1978c). The taste reactivity test II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats. Brain Research, 143, 281-297.

Hankins, W.G., Garcia, J., & Rusiniak, K.W. (1974). Cortical lesions: Flavor illness and noise-shock conditioning. Behavioral Biology, 10, 173-181.

Kiefer, S.W., & Braun, J.J. (1977). Absence of differential associative responses to novel and familiar taste stimuli in rats lacking gustatory neocortex. Journal of Comparative and Physiological Psychology, 91, 498-507.

Kiefer, S.W., & Braun, J.J. (1979). Acquisition of taste avoidance habits in rats lacking gustatory neocortex. Physiological Psychology, 7, 245-250.

Kiefer, S.W., Metzler, C.W., & Lawrence, G.J. (1985). Neocortical involvement in the acquisition and retention of learned alcohol aversions in rats. Alcohol, 2, 597-601.

Lett, B.T. (1985). The painlike effect of gallamine and naloxone differs from sickness induced by lithium chloride. Behavioral Neuroscience, 99, 145-150.

Lorden, J.F. (1976). Effects of lesions of the gustatory neocortex on taste aversion learning in the rat. Journal of Comparative and Physiological Psychology, 90, 665-679.

Lorden, J.F., Kenfield, M., & Braun, J.J. (1970). Response suppression to odors paired with toxicosis. Learning and Motivation, 1, 391-400.

Parker, L. (1982). Nonconsummatory and consummatory behavioral CRs elicited by lithium- and amphetamine-paired flavors. Learning and Motivation, 13, 281-303.

Parker, L. (1984). Behavioral conditioned responses across multiple conditioning/testing trials elicited by lithium- and amphetamine-paired flavors. Behavioral and Neural Biology, 41, 190-199.

Parker, L. (1988). A comparison of avoidance and rejection responses elicited by conditionally and unconditionally aversive tasting solutions. Learning and Motivation, 19, 1-12.

Pfaffman, C., Norgren, R., & Grill, H.J. (1977). Sensory affect and motivation. Annals of the New York Academy of Science, 290, 18-34.

Yamamoto, T., Matsuo, R., & Kawamura, Y. (1980). Localization of cortical gustatory area in rats and its role in taste discrimination. Journal of Neurophysiology, 44, 440-455.

Learned aversions in rats lacking gustatory neocortex: Truly aversive or simply avoided?

by

Missy R. Orr

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ABSTRACT

Rats with gustatory neocortex (GN) lesions were compared to normal rats and rats with control lesions in the acquisition of a conditioned taste aversion to either sucrose (Experiment 1) or sodium chloride (Experiment 2). Rats were adapted to a restricted fluid access schedule and then presented with the tastant. Following consumption of no more than 0.5 ml, rats were immediately intubated with either lithium chloride (trained) or equimolar sodium chloride (untrained). All rats were then tested for taste reactivity to determine the palatability of the conditional taste stimulus. In Experiment 1, control rats rapidly learned an aversion to sucrose but displayed weak negative taste reactivity responses. The GN rats failed to develop aversions relative to the trained control rats but did show a significant reduction in sucrose consumption. Despite the small degree of aversion learning, the trained GN rats displayed virtually no negative taste reactivity. In Experiment 2, naive normal rats displayed rapid taste aversion acquisition to sodium chloride; six of the eight GN rats also acquired a strong aversion. In taste reactivity tests, the trained control group was the only group to display strong negative reactivity; GN rats made no aversive responses. The results demonstrated that GN rats made no negative reactivity responses to

tastes paired with illness, even when conditioning was relatively strong. However, it was shown that GN rats could make negative reactivity responses; GN rats responded aversively to strong quinine solutions in a manner similar to control rats in Experiment 1. In conclusion, GN rats made no negative reactivity responses to tastes previously paired with illness, even when conditioning was relatively strong. Control rats clearly displayed aversive responses to the conditional tastants. Therefore, the avoidance developed by GN rats in a conditioned taste aversion does not entail a hedonic or palatability shift of the conditioned stimulus as it does in control rats.