Learning-based recovery from perceptual impairment in salt discrimination after permanently altered peripheral gustatory input

Ginger Blonde,1,2 Enshe Jiang,1,2 Mircea Garcea,1 and Alan C. Spector1,2

1Department of Psychology and Center for Smell and Taste, University of Florida, Gainesville, Florida; and 2Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, Florida

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Blonde G, Jiang E, Garcea M, Spector AC. Learning-based recovery from perceptual impairment in salt discrimination after permanently altered peripheral gustatory input. Am J Physiol Regul Integr Comp Physiol 299: R1027–R1036, 2010. First published June 16, 2010; doi:10.1152/ajpregu.00843.2009.—Rats lacking input to the chorda tympani (CT) nerve, a facial nerve branch innervating anterior tongue taste buds, show robust impairments in salt discrimination demonstrating its necessity. We tested the sufficiency of the CT for salt taste discrimination and whether the remaining input provided by the greater superficial petrosal (GSP) nerve, a facial nerve branch innervating palatal taste buds, or by the glossopharyngeal (GL) nerve, innervating posterior tongue taste buds, could support performance after extended postsurgical testing. Rats presurgically trained and tested in a two-response operant task to discriminate NaCl from KCl were subjected to sham surgery or transection of the CT (CTx), GL (GLx), or GSP (GSPx), alone or in combination. While initially reduced postsurgically, performance by rats with an intact GSP after CTx + GLx increased to normal over 6 wk of testing. Rats with CTx + GSPx consistently performed near chance levels. In contrast, rats with GSPx + GLx were behaviorally normal. A subset of rats subjected to sham surgery and exposed to lower concentrations during postsurgical testing emulating decreased stimulus intensity after neurotomy showed no significant impairment. These results demonstrate that CTx changes the perceptual nature of NaCl and/or KCl, leading to severe initial postsurgical impairments in discriminability, but a “new” discrimination can be relearned based on the input of the GSP. Despite losing ~75% of their taste buds, rats are unaffected after GSPx + GLx, demonstrating that the CT is not only necessary, but also sufficient, for maintaining salt taste discrimination, notwithstanding the unlikely contribution of the small percentage of taste receptors innervated by the superior laryngeal nerve.


SODIUM IS AN ESSENTIAL ELECTROLYTE that must be constantly replenished from dietary sources. Behavioral studies using rodent models have shown that the chorda tympani (CT) nerve, a branch of the seventh cranial nerve that provides the sole innervation of taste receptor cells in the anterior tongue, is necessary for normal taste-guided behavior related to salts. Both sodium detection and salt discrimination are severely affected by CT transection (CTx) (18, 27, 28, 45, 54, 58, 60). The behavioral findings are not surprising, given that the CT is highly responsive to salts (2, 37, 38, 40, 41) and incorporates a sodium-specific signal via a subpopulation of fibers thought to be the basis for NaCl vs. KCl discriminability, called N-fibers or sodium specialists (8, 15, 30, 37). Amiloride, which blocks epithelial sodium channels (ENaCs) in taste cells, reduces overall electrophysiological responses from the CT and N-fibers to sodium, but not KCl (6, 8, 11, 23, 24, 30, 37), and drops NaCl vs. KCl taste discrimination performance to chance levels in behavioral testing (55). The effects of amiloride treatment are consistent with recent findings demonstrating that sodium taste is severely disrupted in mice with deletion of the α-subunit of the ENaC (9).

Unlike amiloride treatment, however, CTx does not completely eliminate a rat’s ability to discriminate sodium from nonsodium salts. This suggests that the remaining peripheral gustatory input provides some degree of salt discriminability. Indeed, removal of all gustatory input from the seventh cranial nerve by CTx and transection of the greater superficial petrosal (GSP) nerve (GSPx), a branch of the seventh cranial nerve innervating the palate, including the nasoincisor ducts (NIDs) in the rat, has been shown to affect sodium detection and salt discrimination more than CTx alone (1, 17), suggesting that the signal from the GSP does support some degree of salt discriminability. Although not as robustly as the CT, the GSP responds relatively well to NaCl (22, 36) and innervates taste buds that appear to contain ENaCs, as amiloride reduces overall GSP responsiveness to sodium salts (19, 49). However, it is unclear how amiloride is acting on the GSP; a systematic single-fiber analysis of the nerve that includes amiloride treatment has not been conducted, and the work done so far has not found N-fibers such as those found in the CT (50). In contrast to the collective gustatory branches of the seventh cranial nerve (i.e., CT and GSP), the lingual branch of the glossopharyngeal (GL) nerve, which innervates taste buds in the posterior tongue, is unnecessary for sodium detection and salt discrimination (10, 45, 52). It responds only modestly to NaCl and is refractory to the inhibitory effects of amiloride treatment on sodium responses (13, 14, 26, 64; but see Ref. 29). Consistent with the latter finding, in rats the tuning of GL fibers responding to sodium appears to be much less selective than the N-fibers described in the CT (14).

We previously observed that after displaying severe initial impairments in the behavioral discrimination of NaCl from KCl as a function of permanent removal of the taste input from the anterior tongue resulting from CTx, some rats markedly improved their performance over weeks of repeated testing postneurotomy (52). The present study was conducted to 1) confirm this finding, 2) systematically determine the necessity and sufficiency of the anterior tongue taste receptors (innervated by the CT) in subserving salt taste discrimination, and 3) identify the source of the remaining peripheral taste signal that allows the rats to regain near-normal discriminative function after CTx. As will be shown, through experience, animals can regain
discriminative salt taste function following permanent alterations of peripheral gustatory input, but this depends on which gustatory nerves remain intact.

MATERIALS AND METHODS

General Details for Experiments 1 and 2

Apparatus. Training and testing for experiments 1 and 2 took place in a gustometer as described in detail by Spector et al. (53) and modified as described by Blonde et al. (1). Briefly, water-restricted rats were placed in an operant chamber within a sound attenuation enclosure. Small volumes (~5 µl) of fluid stimuli were deposited into a centrally positioned sample spout upon each lick. Taste stimuli were contained in pressurized reservoirs connected via Teflon tubing to solenoid valves and were delivered through the sample spout after it was initially filled at the start of a trial upon the rat licking the sample spout twice within 250 ms. Two reinforcement spouts were positioned on either side of the sample spout; these spouts served to register responses after the rat sampled the stimulus and to deliver fluid reinforcement in the case of a correct response. Cue lights were positioned above both reinforcement spouts. After each trial, the sample spout was rotated over a funnel, rinsed with purified (reverse osmosis-filtered) water (Elix 10, Millipore, Billerica, MA), and blown dry with pressurized air.

Trial structure. As depicted in Fig. 1, each trial began when the rat licked the dry sample spout twice in 250 ms, which ensured that the rat was engaged in spout licking when the stimulus was delivered. During the sample phase, the rat was allowed five licks of the stimulus or 3 s, whichever came first. The decision phase began immediately after the rat had sampled the stimulus, with the sample spout rotating away from the access slot, the house lights extinguishing, and the cue lights illuminating. The rat then had 5 s (referred to as a limited hold) to lick one of the two reinforcement spouts. If the rat responded correctly (i.e., licked the reinforcement spout associated with the stimulus it received during the sample phase), it was allowed up to 10 licks in 8 s from the reinforcement spout. The reinforcement spouts delivered water on control days or 100 µM amiloride hydrochloride on amiloride test days (described below as a part of presurgical testing). If the rat responded incorrectly or failed to respond during the limited hold, it received a 30-s time-out, during which all lights were extinguished and the animal had no access to fluid. The reinforcement or time-out was followed by a 6-s intertrial interval, during which the sample spout was rinsed and dried. The sample spout was then rotated back to the access slot, the house lights were turned on, and the animal could initiate another trial. The rats were allowed to initiate as many trials as possible during each 40-min session.

TRAINING. The rats in experiments 1 and 2 were trained to associate one reinforcement spout with NaCl and the other with KCl (both reagent grade chemicals; Fisher Scientific, Orlando, FL). The spout assignment was counterbalanced: for half of the rats, the left spout represented NaCl, for the other half, it represented KCl. The training and testing schedule is depicted in Table 1.

SPOUT TRAINING. The rats were trained to lick each spout by allowing free access to fluid without contingencies. One 40-min session was devoted to each spout (sample, left reinforcement, right reinforcement), and rats received water ad libitum during the session.

SIDE TRAINING. The rats were trained to associate one reinforcement spout with 0.2 M NaCl and the other with 0.2 M KCl. In each session, only one stimulus was presented via the sample spout, and...
only the reinforcement spout associated with that stimulus was available. The stimulus presented and the reinforcement spout available were alternated each day.

ALTERNATION. Both 0.2 M NaCl and 0.2 M KCl were presented in each session for the first time; both reinforcement spouts were also available for the first time. The rat was required to make a predetermined number of correct responses to a given taste stimulus (e.g., NaCl) before the other stimulus (e.g., KCl) was presented, and this alternation continued throughout the session. This criterion number was reduced systematically, over three sessions, from six responses to two.

DISCRIMINATION TRAINING. In phase I of discrimination training, 0.2 M NaCl and 0.2 M KCl were presented randomly. The probability of receiving either stimulus was 0.5. This phase lasted until every rat performed ≥80% overall on trials with a response.

In phases II and III of discrimination training, the number of stimuli was increased to six to train the animals to respond to all the concentrations with which they were tested: 0.1, 0.2, and 0.4 M NaCl and KCl. The limited hold was systematically reduced, and the time-out was increased. As in phase I, these phases lasted until every rat performed ≥80% overall on trials with a response.

Presurgical testing. Before surgery, the rats were tested on Monday, Wednesday, and Friday with 0.1, 0.2, and 0.4 M NaCl and KCl in control sessions; on Tuesday and Thursday, the salt stimuli were dissolved in 100 μM amiloride, and the reinforcement delivered was 100 μM amiloride. Testing was conducted for 2 wk, for a total of six control sessions and four amiloride sessions.

Postsurgical testing. Postsurgical testing for both experiments began 28–30 days after surgery (see Surgery). For 4 wk, animals were tested on Monday through Friday as described for presurgical control sessions, except for rats that received sham surgery but served as a stimulus intensity control (Sham-Int group), which received concentrations 1.0 log₁₀ unit lower (i.e., 0.01, 0.02, and 0.04 M) as a control to emulate a potential shift in perceived stimulus intensity in the nerve-transected groups. One rat was tested only 4 days during week 2 of postsurgical testing because of suspected illness. During weeks 5 and 6 of postsurgical testing, all rats were tested with amiloride, as during presurgical testing, with amiloride sessions on Tuesday and Thursday. At 3 days after the last day of testing, a water control test was conducted in which all reservoirs were filled with water and arbitrarily assigned as KCl or NaCl to confirm that the performance of the rats relied on chemical cues of the stimuli, rather than extraneous cues.

Histology. At 2–3 days after the water control test, the rats were deeply anesthetized with pentobarbital sodium (60 mg/kg ip) and transcardially perfused with saline and then with 10% buffered formalin. The tongue, soft palate, and NIDs of each rat were removed and stored in 10% buffered formalin. Fungiform papillae were counted as follows: the anterior portion of the tongue from the intermolar eminence to the tip was placed in purified water for 20 min, dipped in 0.5% methylene blue until saturated (∼30 s), and rinsed with purified water; then the epithelium was removed from the underlying tissue and pressed between two slides, and the fungiform papillae and taste pores were counted under a light microscope. The circumvallate papillae and the NIDs were embedded in paraffin and cut into 10-μm sections, which were mounted on slides, stained with hematoxylin and eosin, and evaluated under the microscope. Stained tissues were semiquantitatively analyzed by an observer unaware of the rats’ surgical treatment to determine the success of surgery. The percentage of fungiform papillae with an intact taste pore was used to evaluate the effectiveness of CTx. The number of taste buds in the circumvallate papilla and NIDs was used to evaluate the effectiveness of the transection of the GL (GLx) and GSPx, respectively. Taste buds were counted if it appeared that the taste bud field was not intact (i.e., if there were degenerated taste buds or tissue). After initial histological analysis was complete, the taste buds in the NID in the Sham, CTx, and CTx + GLx groups were counted to determine whether there was evidence of CTx-induced change in this palatal taste receptor field that could account for progressive postsurgical recovery. All tissue sections were coded so that the counters were blind to the surgical condition.

Data analysis. The overall percentage correct for trials with a response was calculated. Individual performance was calculated by averaging percentage correct during control and amiloride sessions (separately for presurgical testing and weeks 5 and 6 of postsurgical testing). Performance during the weeks 1–4 of postsurgical testing was compiled by averaging percentage correct during each week for each animal. For weeks 5 and 6 of postsurgical testing, performance during control sessions was compared, as was performance during amiloride sessions. Group performance was calculated by averaging the overall percentage correct across rats. These data were compared by group using matched t-tests and ANOVAs coupled with Tukey’s honestly significant difference procedure. For these tests, performance by the Sham-Int group was compared directly with performance by other groups, even though rats in the Sham-Int group were receiving lower concentrations in a session. Since the probability of the presentation of either salt was 0.5, chance performance is considered an overall percentage correct of 50%. Group performance was compared with chance levels using one-sample t-tests. The performance of each rat on the water control test was statistically tested for positive differences from chance with the one-tailed normal approximation of the binomial distribution. The conventional α = 0.05 was used as the statistical rejection criterion.

Experiment 1

The purpose of experiment 1 was to determine whether CTx rats would successively improve their disrupted salt discrimination performance with prolonged postsurgical testing as predicted and to determine whether an intact GSP or GL was necessary for such functional recovery in CTx rats.

Animals. Thirty-six male Sprague-Dawley rats, ∼10 wk of age at the start of testing, were studied in two phases. The rats were housed individually in polycarbonate cages during training and testing and in stainless steel hanging wire cages during postsurgical recovery. The room was maintained with a 12:12-h light-dark cycle with temperature automatically controlled with lights coming on at 6:30 AM. Rats had ad libitum access to laboratory rodent chow (Rodent Diet 5001, PMI, Brentwood, MO) in their home cages throughout the experiment. For 1 wk prior to the start of training, oil mash (5.2% parts by weight powdered rodent chow; vegetable oil) was also given to the rats to familiarize them with this diet, which they would receive supplementally during the postsurgical recovery period. During training and testing, water bottles with purified (reverse osmosis-filtered) water (Elix 10, Millipore) were available Friday afternoon after the last testing session until they were removed on Sunday afternoon. On Monday through Friday during training and testing, the rats obtained water as part of their 40-min testing sessions. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Surgery. Surgical groups were balanced in each phase by overall performance, performance relative to each salt, body mass, and gustometer. All rats were anesthetized with an intramuscular injection of ketamine hydrochloride (125 mg/kg body mass) mixed with xylazine hydrochloride (5 mg/kg body mass).

Rats received one of the following surgeries: bilateral CTx, CTx + GLx, CTx + GSPx, or sham surgery (Sham and Sham-Int). For the CTx surgery, the external auditory canal was retracted and the tympanic membrane was punctured. The CT was exposed and transected where it disappears behind the malleus. The ossicles were removed, and the remaining tympanic membrane, as well as the surrounding rim of the ear canal, was cauterized, which generates the production of cerumen, which fills the middle ear to reduce the likelihood of nerve regeneration. For the

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SALT DISCRIMINATION AFTER NEUROTOMY

Experiment 1

Histology. In three rats in the CTx + GLx group, >30% of the fungiform papillae in the anterior tongue contained a taste pore, indicating some level of CT regeneration; data from these animals were not included in the analyses. In one rat in the CTx + GSPx group, 20 taste buds were in the NIDs; however, based on the animal’s performance, it does not appear that the taste buds were sufficient to support discrimination. The statistical outcomes of the analyses were unaffected by whether this rat was included or not; therefore, data from this rat were not discarded. The number of taste buds in the circumvallate papilla for the rats included in the data analysis from the CTx + GLx group was 4.8 ± 1.3 (mean ± SE). The number of taste buds in the NIDs for the rats included in the data analysis from the CTx + GSPx group was 3.0 ± 3.5 (mean ± SE). The percentage of fungiform papillae with an intact taste pore for rats in the CTx groups was as follows: 5.4 ± 0.3%, 0.6 ± 0.3%, and 4.8 ± 0.1% (means ± SE) for CTx, CTx + GLx, and CTx + GSPx, respectively. By comparison, data from our laboratory and reports in the literature indicate that the mean number of taste buds in intact rats, while variable across studies, is ~100 in the NID, 150 in the fungiform papillae (with ~95% of fungiform papillae containing an intact pore), and 400 in the circumvallate papilla (1, 17, 18, 25, 32–35, 51, 52, 57, 60, 62). Most taste bud fields with many taste buds were not counted, but, with the exception of the discarded animals in the CTx + GLx group, all were confirmed as having intact taste nerves. After rats were discarded on histological grounds, the final group sizes were as follows: n = 7 CTx, n = 4 CTx + GLx, n = 8 CTx + GSPx, n = 7 Sham, and n = 7 Sham-Int.

The number of taste buds in the NID was counted for the Sham, CTx, and CTx + GLx groups to determine whether there was evidence of an increased number of taste buds innervated by the GSP in these groups after testing. The number of taste buds was as follows: 100.6 ± 2.31, 91.4 ± 5.41, and 97.7 ± 4.28 (means ± SE) in Sham, CTx, and CTx + GLx, respectively. There was no significant difference between groups, indicating that there was no increase in the number of taste buds in the NID after CTx. Similarly, these means are similar to those found previously by this laboratory in other experiments (1, 52).

Behavioral testing. During presurgical control sessions, all groups were performing at high levels overall (Fig. 2, left). Even after the removal of rats due to apparent partial nerve regeneration, there was no difference in overall percentage correct between groups during presurgical control sessions [F(4,28) = 1.87, P = 0.144]. As expected, the addition of amiloride significantly decreased each group’s overall performance presurgically (P < 0.05 for all groups in t-tests; Fig. 3, left), although performance during amiloride sessions by the Sham and CTx groups was slightly, but significantly, higher than chance (P < 0.05 for both in 1-sample t-tests).

In week 1 of postsurgical testing (i.e., the first 5 sessions), the overall percentage correct for all groups with nerve transections was significantly lower than each group’s presurgical performance levels (P < 0.05 for all groups; Fig. 2, middle). A one-way ANOVA comparing groups during week 1 of postsurgical testing showed a significant effect of group on overall percentage correct [F(4,28) = 24.873, P < 0.001]. Pairwise comparisons using Tukey’s honestly significant difference test
indicated significantly poorer performance by all nerve-transected groups compared with Sham rats \( (P < 0.001 \text{ for all groups}) \). Additionally, CTx and CTx + GLx rats performed at similar levels \( (P > 0.05) \). The performance of CTx + GSPx rats was at chance levels \( (P = 0.781) \) and significantly lower than that of CTx and CTx + GLx rats \( (P < 0.021) \), suggesting that the GSP contributed to performance in this discrimination. Interestingly, the performance of Sham-Int rats was similar to that of Sham animals, despite testing with concentrations that were 1.0 \( \log_{10} \) unit lower \( (P > 0.05) \). This group was included as a control for a likely attenuation in stimulus intensity that occurs after CTx \((28, 46, 58)\). We lowered the stimulus concentrations by a \( \log \) unit during postsurgical testing in a subset of rats subjected to sham surgery to test whether the initial drop in performance following nerve transection could have been simply due to a weakened stimulus. However, the relatively good performance of the Sham-Int group suggests that the impairment in rats with nerve transections was not simply due to a reduction in stimulus intensity after surgery.

By week 2 of testing, performance of the CTx and CTx + GLx groups significantly improved compared with the previous week \( (P < 0.035 \text{ and } P < 0.004, \text{ respectively}) \), although performance of both groups remained below presurgical levels \( (P < 0.05 \text{ for both}) \). Performance of CTx and CTx + GLx groups continued to improve slightly throughout postsurgical testing. These two groups were never significantly different from each other. The CTx + GLx group reached presurgical performance levels by the end of week 4 of postsurgical testing \( (P > 0.2 \text{ in a paired } t\text{-test}) \). Performance of CTx + GSPx rats, on the other hand, remained at or near chance levels throughout postsurgical testing based on one-sample \( t\)-tests \( (P > 0.05 \text{ for postsurgical weeks 1–4; } P = 0.02 \text{ during postsurgical control sessions; Figs. 2 and 3}) \). As with presurgical testing, all groups performed at or near chance levels when tested with amiloride (Fig. 3, right). One-sample \( t\)-tests revealed that performance of the Sham group was again significantly >50% overall during amiloride sessions \( (P < 0.03) \), but performance of all groups was clearly impaired relative to control sessions \( (P < 0.05 \text{ for all groups}) \) and was close to chance levels. Performance of individual rats during the water control test ranged from 43% to 52% overall; binomial analyses showed that no rat performed significantly above chance levels \( (P > 0.05) \).

**Experiment 2**

**Histology.** All the tissues were determined to have a taste bud count appropriate for the surgical condition of the animal. As such, no animals were removed from analysis. The number of taste buds in the circumvallate papilla for the GSPx + GLx group was \( 2.1 \pm 2.3 \) (mean \( \pm \text{SE} \)). The number of taste buds in the NIDs was \( 1.9 \pm 2.3 \) and \( 2.0 \pm 2.2 \) (means \( \pm \text{SE} \)) for the GSPx and CTx + GSPx groups, respectively. As in experiment 1, taste bud fields with many taste buds were not counted, but all were confirmed as having intact taste nerves. Final group sizes were as follows: \( n = 6, n = 6, \) and \( n = 6 \) for Sham, GSPx, GSPx + GLx, respectively.

**Behavioral testing.** Presurgically, all groups performed at high levels overall, with no significant difference of overall percentage correct between the groups in a one-way ANOVA \( (F(2,15) = 1.84, P > 0.05; \text{ Fig. 4, left}) \). The addition of amiloride during presurgical testing significantly reduced per-
formance to chance levels in all groups in a one-sample t-test ($P < 0.05$ for all groups; Fig. 5, left).

Postoperatively, there was no effect of GSPx or GSPx + GLx: neither surgical group differed from its presurgical performance levels ($P > 0.05$ for both groups in paired t-tests) or from the Sham group during postsurgical testing, with no effect of group in a one-way ANOVA [$F(2,15) = 2.03, P > 0.05$; Fig. 4, middle]. Over the course of the 6 wk of postsurgical testing, all the groups improved beyond their presurgical levels. Amiloride reduced overall performance postsurgically as before to near chance levels; in the Sham group, performance was significantly above chance levels during the amiloride sessions ($P < 0.02$ in a 1-sample t-test) but was clearly impaired compared with control sessions ($P < 0.005$ in a paired t-test; Fig. 5, right). During the water control test, performance of individual rats was between 44% and 53%, and no value was significantly higher than chance based on one-tailed normal approximation of the binomial distribution ($P > 0.05$).

**DISCUSSION**

Rats, one of the most common animal models in gustatory research, display a remarkably keen ability to detect sodium-containing compounds and to discriminate such compounds from other stimuli on the basis of taste (7, 16, 21, 27, 45). A great deal of taste research has focused on the peripheral and central neural mechanisms subserving salt discriminability in rodent models. The results of the present study add to the existing literature in two important ways. First, in addition to its known necessity, our results reveal the functional sufficiency of the CT to support absolutely normal NaCl vs. KCl discrimination by the rat, although this branch of the facial nerve innervates only 15% of the total oropharyngeal taste buds. The necessity of the input from the CT in salt discrimination performance requires qualification, however, which brings us to the second important finding of our study. Animals with permanent CTx can regain normal discriminative function with prolonged postsurgical testing, but only if the GSP, the branch of the facial nerve innervating palatal taste buds, remains intact; the GL alone is unable to support any recovery of function. The implications of these two findings are discussed in turn below.

**Functional Sufficiency of the CT in Salt Discrimination**

It has been known for some time that the CT is necessary for normal discrimination of sodium from nonsodium salts; CTx severely impairs salt discrimination but does not abolish it (27, 51, 52, 54, 60). Transection of the GL, leaving the CT and GSP intact, has been shown to have no effect in a range of salt-based gustatory tasks, including sodium detection and recognition, and salt discrimination (1, 10, 32, 52). Thus the ninth cranial nerve is unnecessary for this discrimination, indicating the sufficiency of the gustatory branches of the seventh cranial nerve in maintaining the ability to discriminate between NaCl and KCl. Elimination of the gustatory input from the facial nerve (i.e., CTx + GSPx) virtually abolishes salt discrimination, a finding confirmed in the present report, and markedly elevates NaCl detection thresholds (1, 17). Collectively, these findings demonstrate the necessity and the sufficiency of the seventh cranial nerve in salt discrimination.

Until the present study, the relative contribution of the two gustatory branches of the facial nerve to the ability to discriminate between salts had not been tested. More specifically,
would GSPx, alone or in combination with GLx, also lead to impairments in the ability to discriminate between NaCl and KCl, as seen after CTx alone? In experiment 2, the GSPx group showed no impairment in this task, suggesting that although the GSP provides some relevant input for differentiating NaCl from KCl in combination with the CT (as shown by the CTx + GSPx group), the nerve itself is not necessary for normal discriminability. Similarly, in experiment 1, the CTx + GLx group showed the same initial decrease in performance as the CTx group, indicating that the GSP is insufficient to maintain normal performance in this case. In contrast to the GSP, the CT is both necessary and sufficient to allow the rat to discriminate NaCl from KCl. In the CTx group, in experiment 1 here (Fig. 2) and in other studies (27), performance was impaired compared with Sham rats. Yet, when animals were deprived of gustatory input from the GL and GSP (i.e., GSPx + GLx group; Fig. 4), the ability to discriminate NaCl from KCl remained unperturbed. Accordingly, the experiments conducted here have importantly contributed to the completion of the matrix defining the anatomic requirements in the rat gustatory periphery for the maintenance of salt taste discrimination, as depicted in Table 2, and thus provide a context for evaluating various models of taste quality coding at least with respect to salt. The CT and its associated central circuits possess everything necessary for normal salt taste discrimination as measured here, and the GL, along with its associated central circuits, does not.

One caveat regarding these conclusions is that the contribution of the superior laryngeal nerve (SLN), a branch of the 10th cranial nerve, which in rodents innervates taste buds in the laryngeal epithelium (35, 63), in maintaining performance under some nerve transection conditions cannot be entirely dismissed. It is clear that the presence of the SLN alone is entirely insufficient to allow any salt discriminability, as shown by the virtual chance performance of the CTx + GSPx group. Based on the position of the taste buds it innervates and its general response properties, it has been suggested that the primary role of the SLN is the protection of the airway (5).

Recovery of Salt Discrimination Function After Permanent CTx

Surprisingly, the impairment in salt discrimination after permanent loss of input from the anterior tongue taste receptors (i.e., CTx), while severe, can be reversed with further testing. Over time, as seen previously (52) and replicated here, performance of this task was significantly improved in some rats, even after verification of CTx. Here, we show that this recovery depends on the GSP, and not the GL. Over the course of postsurgical testing, performance of CTx + GLx rats was similar to CTx rats, suggesting that the GL is not necessary to support the recovery of discriminative performance in the absence of the CT. The GSP, on the other hand, is sufficient in this case and is necessary as well. Rats with both of the gustatory branches of the seventh cranial nerve transected, leaving only the ninth cranial nerve (and the SLN) intact, performed at nearly chance levels.

The necessity and sufficiency of the GSP to support recovery of salt discrimination performance in the absence of the CT are corroborated by performance during amiloride test days. After showing marked improvement in the discrimination, despite lacking the input of the CT, performance of rats in the CTx, CTx + GLx, and CTx + GSPx groups dropped to chance levels when amiloride was added to the tastants (Fig. 3). Clearly, the recovery of function displayed by these groups relies on the amiloride-sensitive salt taste transduction pathway. The CT and GSP innervate taste buds that contain amiloride-sensitive taste receptor cells thought to be the basis for the discrimination between sodium and nonsodium salts (6, 11, 12, 23, 24, 37, 45, 55, 65). The GL, however, does not appear to innervate functional amiloride-sensitive taste cells (12, 13, 26; but see Ref. 29), and as such it would seem that the nerve is unable to provide signals that discriminate sodium from nonsodium salts, thus precluding competent performance of this task. Moreover, based on the collective results of nerve transection studies in the literature, the input of the seventh cranial nerve is critical for all taste quality discriminations, at least those tested to date, whereas the input of the ninth nerve is not (51, 62).

The exact mechanism by which rats relearn the discrimination remains to be determined. Histology showed that regeneration of the CT did not occur, so although it has been shown that a regenerated CT can support the behavior (27), it is not the case here. Given that CTx + GSPx, as well as the application of amiloride, eliminated the ability to discriminate between salts in those groups that were able to regain presurgical levels of performance, the role of taste, rather than trigeminal or nonoral cues (which would be present for all groups), is strongly implicated in this process. Moreover, the fact that the CTx and CTx + GLx rats displayed initial postsurgical deficits in their ability to discriminate between NaCl and KCl similar to rats in a prior study (27) presurgically trained to discriminate between NaCl and KCl taste and first tested postsurgically 62 days after CTx (corresponding to week 5 of postsurgical testing here) demonstrates that the recovery of function observed here is not due simply to some physiological or anatomic change occurring over time; postsurgical behavioral experience in the task is also necessary. While changes that allow for the improvement in performance are likely occurring somewhere along the gustatory neuraxis, these changes appear to require postsurgical experience.

One possibility is that the initial drop in postsurgical performance is attributable to a decline in perceived stimulus intensity after CTx, making it difficult at first for the rat to distinguish between the two salts. The 1.0 log$_{10}$ unit decrease in stimulus concentrations for the Sham-Int rats was designed to emulate the lowered taste sensitivity after nerve transection. One might expect, then, that the nerve-transected groups would
show a level of performance similar to that of the Sham-Int rats, if the only effect of nerve transections was to decrease the perceived intensity of the stimuli. However, the performance of the Sham-Int group was not significantly different from the Sham group, unlike rats in the groups with nerve transections. The level of performance was, however, slightly, if not significantly, lower in the Sham-Int than in the Sham rats, and thus a decline in perceived stimulus intensity might be partially responsible for the drop in performance for the groups with nerve transections, but clearly other factors are at play.

It is also possible that the respective taste qualities of NaCl and/or KCl were changed by CTx but that the two tastants could still be discriminated, requiring relearning. For example, when intact rats were first trained to discriminate quinine from KCl and then tested on a NaCl vs. KCl task, their performance initially dropped to near chance but progressively improved with further testing (56). It appears that a similar phenomenon occurred in the CTx and CTx + GLx groups postsurgically. The change in the perceptual nature of the signals after CTx need not be qualitative per se but, rather, might be related to neurotomy-induced alterations in the temporal rise and decay times of the intensity of the sensations. Similarly, perhaps the oral origin of stimulation (i.e., anterior tongue vs. posterior tongue vs. palate) represents a salient feature of the presurgically trained stimuli. Accordingly, postsurgical changes in these aspects of the signals coupled with the known changes in intensity might collectively be sufficient to constitute the presentation of somewhat novel stimuli requiring retraining on the task.

Concluding remarks. CTx clearly causes some change in the perceptual nature of NaCl or KCl (or both) that is reflected in a consistent and robust impairment in salt discrimination performance that is not seen with any other gustatory nerve transection. Such findings highlight the primacy of the CT in salt taste. The exact nature and extent of the perceptual change in salt perception as a function of CTx remain unclear, but the changes do not render the two salts entirely indiscriminable: rats can still discriminate between NaCl and KCl, but only after a period of continued postsurgical testing and only if the GSP is left intact. The fact that normal salt taste discrimination function can be restored through behavioral experience after sometimes massive denervations (e.g., CTx and GLx eliminates ~75% of the total taste buds) underscores the remarkable capacity of rats to adapt to alterations in peripheral gustatory input through learning.

Perspectives and Significance

Since the pioneering work of Curt Richter on the phenomenon of salt appetite (42–44), taste science has made significant advances to discern critical features of the underlying neural machinery that allow rodents and, likely, other omnivorous and herbivorous species, to identify sodium salts. There are sufficient converging lines of evidence that, in the rat, the ability to identify sodium relies heavily on the CT, which, as shown here, appears to be both necessary and sufficient to allow the animal to discriminate between NaCl, the archetypal sodium salt, and KCl, a common and physiologically relevant nonsodium salt (59). The ENaC is an essential receptor, and its selective permeability characteristics underlie the sodium-specific nature of the N-units, sometimes referred to as sodium specialists, found in the CT. The behavioral results presented here coupled with those in the literature now give free license to researchers trying to understand the central coding of sodium taste to focus specifically on the anterior tongue taste receptors without concern about the other taste receptor fields. The CT and the anterior tongue taste receptors it innervates appear to contain everything necessary to allow for competent salt discrimination, notwithstanding the unlikely contribution of the SLN. Also, the presence or absence of sodium in a sampled taste stimulus appears to be signaled in the periphery in a labeled-line fashion. That is, if the N-units fire, sodium is present; if they do not fire, sodium is not present. The fact that N-units have yet to be identified in the GSP (50) but, as shown here, the GSP can support discrimination between NaCl and KCl does raise some questions regarding the absolute necessity of N-units. A complete resolution to this issue, however, awaits a single-fiber analysis of palatal responses to salt stimuli that includes amiloride treatment. With that caveat in mind, it would be worthwhile for researchers to continue to exploit what is known about the sufficiency of the CT as well as the necessity of the ENaC taste receptors in behavioral responses to sodium by selectively stimulating the anterior tongue with salts and other compounds before and after amiloride treatment and recording single-cell activity at various levels of the gustatory neuraxis to help define which neurons are likely critical in subserving the discrimination between sodium and nonsodium salts. Such studies have already led to some insight (3, 4, 20, 31, 39, 47, 48, 61), but more work is needed to help distinguish between various taste quality coding models in the central gustatory system.

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REFERENCES


