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REWIRING THE GUSTATORY SYSTEM: SPECIFICITY BETWEEN NERVE AND TASTE BUD FIELD IS CRITICAL FOR NORMAL SALT DISCRIMINATION

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Abstract

Forty years have passed since it was demonstrated that a cross-regenerated gustatory nerve in the rat tongue adopts the stimulus-response properties of the taste receptor field it cross-reinnervates. Nevertheless, the functional consequences of channeling peripheral taste signals through inappropriate central circuits remain relatively unexplored. Here we tested whether histologically confirmed cross-regeneration of the chorda tympani nerve (CT) into the posterior tongue in the absence of the glossopharyngeal nerve (GL) (CT-PostTongue) or cross-regeneration of the GL into the anterior tongue in the absence of the CT (GL-AntTongue) would maintain presurgically trained performance in an operant NaCl vs. KCl taste discrimination task in rats. Before surgery all groups were averaging over 90% accuracy. Oral amiloride treatment dropped performance to virtually chance levels. During the first week after surgery, sham-operated rats, GL-transected rats, and rats with regenerated CTs displayed highly competent discrimination performance. In contrast, CT-transected rats were severely impaired (59% accuracy). Both the CT-PostTongue and the GL-AntTongue groups were impaired to a similar degree as CT-transected rats. These initially impaired groups improved their performance over the weeks of postsurgical testing suggesting that the rats were capable of relearning the task with discriminable signals in the remaining taste nerves. This relearned performance was dependent on input from amiloride-sensitive receptors likely in the palate. Overall, these results suggest that normal competence in a salt discrimination task is dependent on the taste receptor field origin of the input as well as the specific nerve transmitting the signals to it associated circuits in the brain.

Keywords

Taste; Nerve Regeneration; Cross-Reinnervation; NaCl; KCl; Taste Perception

INTRODUCTION

The rodent gustatory system represents an excellent experimental platform for studying neural plasticity and its functional concomitants. Taste bud cells have limited life spans on the order of about 10 days and are routinely replaced by perigemmal progenitor cells (Beidler and

Smallman, 1965; Farbman, 1980). This means that new synapses are constantly being formed. Moreover, taste buds, which degenerate upon interruption of the nerve supply, reappear when a transected lingual taste nerve regenerates and reinnervates its native receptor field (Barry and Frank, 1992). Notably, for the most part, taste functions that are disrupted upon nerve transection, are restored once the nerve successfully reinnervates its normal epithelial region (Barry et al., 1993; Cain et al., 1996; St John et al., 1995; King et al., 2000; Kopka et al., 2000; Kopka and Spector, 2001; Yasumatsu et al., 2003; Geran et al., 2004). For the chorda tympani nerve (CT), this recovery of function occurs despite the seemingly permanent anatomical alterations in the gustatory system following regeneration including: decreases in the number of anterior tongue taste buds and their volume (Shuler et al., 2004), decreases in the number of myelinated fibers in the regenerated CT coupled with decreased number of cell bodies in the geniculate ganglion (Cain et al., 1996), and reduced volume and density of the CT terminal projection field in the nucleus of the solitary tract (Barry, 1999).

One of the truly remarkable characteristics of the peripheral gustatory system, at least in rodents, is that the lingual taste nerves can be surgically cross-wired (Oakley, 1967a; Oakley, 1967b; Oakley, 1995; Ninomiya, 1998; Smith et al., 1999; King et al., 2008). This provokes questions regarding the functional consequences of cross-regeneration. Relatively little has been done to examine this behaviorally. Several decades ago, Oakley (1969) demonstrated that rats surprisingly displayed normal preference-aversion functions in long-term two-bottle tests after the glossopharyngeal nerve (GL), which normally innervates taste buds in the posterior tongue, was cross-regenerated into the anterior tongue on one side and the remaining lingual taste receptor fields were left denervated. These tests, however, are not optimal for testing gustatory function. In fact, completely denervating the taste buds on the tongue has limited effects on such measures (see Spector, 2003).

In order to approach the question of whether animals with cross-regenerated taste nerves are functionally competent, it is critical that two conditions be met. First, a taste-related behavior that is unequivocally disrupted by transection of one lingual gustatory nerve but not by the other must be used in the experimental design. Second, any transection-induced disruptions in behavior must recover upon normal regeneration of the nerve. Recently, King et al. (2008) conducted an experiment that satisfied these two conditions. Gaping, a hallmark taste-elicited oral motor rejection response, is severely disrupted by GL but not CT transection (Travers et al., 1987; Grill et al., 1992; King et al., 1999; King et al., 2000). The response is restored upon normal regeneration of the GL (King et al., 2000). King et al. (King et al., 2008) found that quinine-induced gaping in rats was normal in rats that had the posterior tongue taste buds cross-reinnervated by the CT with the anterior tongue taste buds left denervated. In contrast, rats that had the anterior tongue taste buds reinnervated by the GL with the posterior tongue taste buds left denervated were severely impaired. Thus, in this particular case, the receptor field reinnervated appeared to be the critical variable, not the nerve transmitting the signals to the brain.

In this paper, we focus on the effects of cross-regeneration of the CT and GL on salt taste discrimination performance in rats. Operantly conditioned performance on such tasks, while high presurgically, is severely disrupted by CT transection, but is entirely unaffected by GL transection (Spector and Grill, 1992; St John et al., 1995; Kopka et al., 2000). When the CT regenerates, performance returns to normal. The experiment reported here examined whether the recovery of function upon reinnervation of the anterior tongue taste buds is dependent on the reinnervating nerve, the reinnervated field of taste receptors, or both.

RESULTS

Before surgery all of the rats performed the salt discrimination task extremely well and there were no differences among the groups in their percentage correct across all trials collapsed across stimuli and concentrations ($F(6,26)=.46$, $p=.835$; Figure 1). As expected, when the epithelial sodium channel (ENaC) blocker amiloride, which suppresses the sodium-specific taste transduction pathway in rodents, was used as the solvent, performance dropped to levels at or near chance and there were no differences between the groups ($F(6,26)=.72$, $p=.67$; Figure 2). Because there were no obvious differences between the performance of the CTr + GLx and the CTr + GLr groups either presurgically or postsurgically on any of the behavioral measures, they were combined into a single sample for the statistical analysis above and those subsequently reported, except where noted for histological analyses.

During the first week of postsurgical testing, there were significant differences between the levels of performance exhibited by the various surgical groups ($F(6,26)=20.9$, $p<.001$; Figure 3). All rats that had a histologically confirmed bilateral CT transection included in their surgical treatment displayed the expected impairment in salt discrimination performance. The overall percentages correct collapsed across stimuli and concentration for the CTx and CTx + GLr (unil) + GLx groups were significantly lower than those for the SHAM, GLx, and CTr groups (all p -values $<.001$), which, in turn, did not significantly differ among each other. Thus, bilateral transection of the GL had no effect on salt discrimination performance and normal regeneration of the CT to the anterior tongue resulted in complete recovery of function.

In striking contrast to the CTr group, neither cross-regenerated group could perform the discrimination better than the CTx group during the first week of postsurgical testing. Both cross-regeneration groups had significantly lower performance scores compared with the SHAM, GLx, and CTr groups (all p -values $\leq .001$).

With continued postsurgical testing, most of the animals in all groups were able to increase performance, but the cross-regenerated groups never performed better statistically than the CTx group. This is evident by the fact that performance in each group, with the exception of the CTx + GLr(unil) + GLx ($p=.213$), during the postsurgical control sessions (weeks 5 & 6) improved significantly compared with that observed during the first postsurgical week (all p -values $< .047$; Figure 4). Indeed, although the presence of a significant difference was detected ($F(6,26)=3.65$, $p=.009$) when an ANOVA was conducted comparing the performance during the postsurgical control sessions (weeks 5 & 6) of all of the groups, post-hoc comparisons conducted with the FDR procedure indicated that only the CTx + GLr(unil) + GLx group differed significantly from the SHAM rats. We also conducted a repeated measures ANOVA for overall performance in each group across postsurgical weeks 1 through 4 (Figure 5) and found significant increases (all p -values $.05$) except for the CTx + GLr(unil) + GLx ($p=.103$). Overall, this suggests that most CTx and cross-regenerated animals were able to “relearn” the discrimination with the remaining intact gustatory input.

Whatever the exact neural basis of the “relearned” discrimination is, it depended on functioning ENaCs because the use of amiloride as the solvent dropped performance in all groups to levels at or near chance with no differences detected between the groups ($F(6,26)=.67$, $p=.68$; Figure 6).

Finally, no animal performed significantly greater than 50% (chance) during the water test. This confirms that the animals were responding on the basis of the chemical nature of the stimulus and not extraneous cues.

Figure 7 displays the taste bud counts of the rats included in the statistical analysis of salt discrimination performance described above. Histological evaluation of the anterior tongue

revealed that animals that had the anterior tongue denervated had very few if any intact taste pores (overall $F(6,26)=59.9$, $P<.001$). Importantly, animals that had the anterior tongue reinnervated by either the CT or GL had similar numbers of taste buds to sham-operated rats. Indeed, there was no significant difference in the number of anterior tongue taste pores observed in the GL-AntTongue group compared with the SHAM animals.

For the analysis of the posterior tongue taste buds, the CTr + GLx and the CTr + GLr rats had to be split into separate groups because in one case the posterior tongue was denervated and in the other it was not (in the analyses above they were combined). As expected, an overall ANOVA indicated significant differences in the number of taste buds in the circumvallate papilla (CV) across the groups ($F(7,25)=45.7$, $p<.001$). Animals without any nerve innervating the posterior tongue had virtually no taste buds in the circumvallate papilla. There were no significant differences in the number of CV taste buds in groups that had an intact or regenerated GL on both sides with the exception that the CTX group had significantly fewer CV taste buds than the SHAM group ($p=.012$). As expected, the group with the GL transected on one side and regenerated on the other (CTx + GLr(unil) + GLx) had roughly half the number of taste buds seen in the SHAM group ($p<.001$). The cross-regenerated group that had the CT routed to the posterior tongue (CT-PostTongue) also had about half the number of CV taste buds compared with SHAM animals ($p<.001$). An analysis of the number of total taste buds in the foliate papillae roughly emulated that conducted on the CV ($F(7,25)=31.6$, $p<.001$), except that the number of foliate taste buds in the CTX rats was not significantly different from that observed in the SHAM group, but was different from that observed in the CTr + GLr rats ($p=.03$). Although the number of taste buds in the CV and foliate papillae in the CT-PostTongue group were significantly reduced relative to SHAM operated controls, the total number of posterior tongue taste buds reinnervated were more than double what the chorda tympani normally innervates in the anterior tongue.

DISCUSSION

The results of this experiment unequivocally confirm that loss of neural input from the chorda tympani nerve, which innervates the taste buds of the anterior tongue, severely compromises salt taste discrimination in the rat and in some other rodent models, and that transection of the glossopharyngeal nerve, innervating the posterior tongue taste receptors accounting for 60% of the total population, is entirely without effect (e.g., (Sollars and Bernstein, 1992; Spector and Grill, 1992; Breslin et al., 1993; Barry et al., 1993; O'Keefe et al., 1994; St. John et al., 1995; Markison et al., 1995; Breslin et al., 1995; Frankmann et al., 1996; St John et al., 1997; Smith et al., 1999; Kopka et al., 2000). Moreover, salt discrimination performance completely recovers upon reinnervation of the anterior tongue from the regenerated chorda tympani nerve (Barry et al., 1993; St. John et al., 1995; Kopka et al., 2000; Yasumatsu et al., 2003). These findings provide the context for the interpretation of the cross-reinnervation results. Specifically, it appears that initial recovery of function in this task depends on the chorda tympani nerve reinnervating its appropriate taste receptor field in the anterior tongue. If the glossopharyngeal nerve reinnervates the anterior tongue taste buds in the absence of the chorda tympani nerve, or if the chorda tympani nerve reinnervates the posterior tongue taste buds in the absence of the glossopharyngeal nerve, rats display impairments in salt discrimination equivalent to that observed after permanent chorda tympani nerve transection. Finally, although many of the modifications of the peripheral gustatory system conducted here resulted in severe impairments in salt taste function as assessed in the first week of postsurgical testing, most of the animals were able to make use of the remaining neural input to regain competence in the task. Each of the above findings has implications regarding the functional organization of the gustatory system and the capacity of animals to adapt to altered peripheral taste input.

In pioneering studies, Oakley (1967a; 1967b) showed that when the CT reinnervated the posterior tongue instead of the anterior tongue, it adopted the electrophysiological response properties of the normal GL – it responded well to quinine and saccharin and poorly to NaCl. When the GL reinnervated the anterior tongue, it adopted the response properties of the normal CT – it responded well to NaCl and poorly to quinine and saccharin. Oakley concluded that it was the characteristic chemosensitivity of taste receptor cells of a given region of the tongue that dictated the taste response properties of the nerve innervating them, not the converse. Nejad and Beidler (Nejad and Beidler, 1994) reached a similar conclusion when they routed the greater superficial petrosal (GSP), which normally innervates palatal taste receptors, into the anterior tongue, and, in other animals, routed the CT into the palate. Under intact conditions the GSP responds exceptionally well to sucrose placed on the palate and only modestly to NaCl, whereas the CT responds well to NaCl placed on the anterior tongue and poorly to sucrose. The cross-regenerated nerves adopted the chemosensitivity of the receptor field they were re-innervating. Smith and colleagues (Smith et al., 1999) found the same to be true for the relative expression of gustducin and the a-blood group antigen, a cell surface carbohydrate, in taste buds cells. Thus, in these experiments, the phenotypes of the taste receptor cells appeared to be regionally dependent irrelevant of the taste nerve innervating them, at least in rats.

Our current working hypothesis underlying the initial impairment in discrimination performance observed in this study is diagrammed in Figure 8. We believe that initial postsurgical salt discrimination performance is not supported by the chorda tympani nerve when it is cross-wired to the posterior tongue due to an insufficient discriminative signal arising from the posterior tongue taste receptor cells. Normally, in the rat peripheral gustatory system there are single units that respond selectively to sodium (and Li⁺) salts; these are referred to in the literature as N-units or sodium specialists (Frank et al., 1983; Ninomiya and Funakoshi, 1988; Lundy, Jr. and Contreras, 1999; Breza et al., 2006). The sodium response in these neurons is severely suppressed by the topical application of the epithelial sodium channel (ENaC) blocker, amiloride, to the anterior tongue, suggesting that Na-selective ENaCs or some other amiloride-sensitive channels with similar cation selectivity in taste receptor cells confer the N-units with their response specificity (Ninomiya and Funakoshi, 1988; Lundy, Jr. and Contreras, 1999). Another class of units responds more broadly to salts and acids (and sometimes quinine). These have been called H-units, A-units, E-units or electrolyte generalists (Frank et al., 1983; Ninomiya and Funakoshi, 1988; Frank, 1991; Lundy, Jr. and Contreras, 1999; Breza et al., 2006) and apparently are associated with ion channels serving as receptors that display poor cation selectivity and amiloride insensitivity. Both the specialist and the generalist types can be found in the chorda tympani nerve and thus both receptor types appear to be present in the anterior tongue. The salt responsive fibers in the rat glossopharyngeal nerve, however, are only of the electrolyte generalist type (Frank, 1991). This is consistent with the failure of amiloride to alter the sodium responsiveness of the rat glossopharyngeal nerve when it is applied to the posterior tongue (Formaker and Hill, 1991; Gilbertson and Zhang, 1998; Kitada et al., 1998). Accordingly, there does not appear to be taste receptor cells in the posterior tongue, functionally connected to glossopharyngeal nerve fibers, that differentially respond to sodium vs. non-sodium salts. Thus, when the chorda tympani nerve cross-reinnervates the posterior tongue taste buds, we hypothesize that there is no signal provided by those taste receptor cells that differentiates between NaCl and KCl (Figure 7B), leading to poor performance initially by rats in the CT-PostTongue group. The reason performance does not drop to chance is because some discriminable signals appear to be provided by the GSP, which has been shown to decrease its responsiveness to NaCl following amiloride treatment.

This assumes that, as suggested by Oakley (1967a; 1967b), the response properties of the posterior tongue taste receptor cells do not change as a function of chorda tympani cross-reinnervation. However, in mice, Ninomiya (1998) found that when the CT cross-reinnervated the posterior tongue, it maintained its amiloride sensitivity, whereas when the GL cross-

reinnervated the anterior tongue, its responses to NaCl stimulation were not influenced by amiloride treatment. In this case, the cross-regenerated nerve did not adopt the response profile of the taste receptor field it was reinnervating. Ninomiya (1998) suggested that the cross-regenerated CT fibers were able to appropriately match with the few amiloride-sensitive taste receptor cells that might be present in the posterior field. Thus, it remains to be determined whether the sodium responses in the rat glossopharyngeal nerve cross-reinnervating the anterior tongue are suppressible by amiloride treatment.

In the case of the glossopharyngeal nerve cross-reinnervating the taste buds of the anterior tongue, discriminable signals are likely provided by the taste receptor cells potentially allowing the animal to distinguish between NaCl and KCl. However, we hypothesize that information is channeled through neural circuits that do not contribute to the processing of perceptual quality (Figure 7C). Indeed, there is growing evidence suggesting that, in rats, the neural input of the gustatory branches of the seventh cranial nerve are necessary and sufficient for sensory identification of taste compounds and discrimination between them regardless of their chemical or perceptual class. The ninth cranial nerve does not appear to be necessary nor sufficient to maintain performance in this domain of taste function, at least in the rat model (see St John and Spector, 1998; Spector, 2003).

The failure of either cross-regeneration condition to lead to initial recovery of salt discrimination performance is to be contrasted with the ability of the chorda tympani nerve to support quinine-induced gaping when rewired to reinnervate the taste buds of the posterior tongue in the absence of the glossopharyngeal nerve (King et al., 2008). When the glossopharyngeal nerve is rewired to reinnervate the anterior tongue taste buds in the absence of the chorda tympani nerve, quinine-induced gaping remains severely attenuated. King et al. (King et al., 2008) suggested that there is a population of afferents in the chorda tympani that are competent at triggering gapes in response to quinine if adequately stimulated. Because the quinine signal arising from the anterior tongue taste buds, a region that has low expression of T2Rs, thought to be the primary receptors for bitter ligands (Adler et al., 2000), is weak relative to that in the posterior tongue, the chorda tympani nerve is generally unable to support much unconditional gaping to the stimulus in the absence of the glossopharyngeal nerve (even in the presence of the greater superficial petrosal nerve innervating the palatal taste buds). However, when the chorda tympani innervates the posterior tongue taste receptor cells, which generally respond well to quinine and in which T2R expression is normally high, then the signal is sufficiently strong to trigger unconditional gapes to the stimulus. Although the glossopharyngeal nerve is normally connected to circuits that are capable of generating gapes in response to quinine, when the nerve is rewired to innervate the anterior tongue taste buds, the signal is too weak to be effective. Regardless of the interpretation of these experimental outcomes, it is clear that the effectiveness of cross-regeneration to restore lost taste function depends on the region of the tongue reinnervated, the reinnervating nerve and its associated central connections, and the nature of the taste-related behavior measured.

Although animals that had the chorda tympani nerve permanently transected or rerouted to the posterior tongue all displayed poor performance in the salt discrimination task during the first week of postsurgical testing, many of the animals in these groups steadily improved their performance with continued postsurgical testing. This suggests that most animals are able to make use of the remaining neural input to relearn the discrimination. Whatever the source of these signals, the discrimination depended on amiloride-sensitive channels given that amiloride adulteration of the stimuli dropped performance to virtually chance levels. The presence of amiloride sensitive channels in palatal taste bud cells implicates the neural input of the greater superficial petrosal as the primary basis for the relearned behavior (Gilbertson and Zhang, 1998; Sollars and Hill, 1998), although the cation selectivity of the GSP fibers that decrease responsiveness to NaCl upon amiloride treatment remains unknown.

If a discriminable signal was present postsurgically in the cross-regenerated or CT-transected groups, why was performance so disrupted initially after surgery? The most parsimonious answer is that there is a difference between the taste perception of NaCl, KCl, or both after these surgical manipulations. Presurgical training occurred with an intact gustatory system and the animals learned to discriminate between two distinct taste signals representing different qualitative perceptions. After CT transection or rerouting, the nature of those discriminable signals changed presumably changing the nature of the qualitative taste perception generated by the salt stimuli. This qualitative change need not be, nor likely is, a complete alteration of the basic tastes of these stimuli (e.g., say from “salty” to “sweet”). Indeed, the change need not be qualitative per se, but might deal with alterations of the perceived intensity of the stimuli, the rise and decay times of the sensation, or even simply the region of the oral cavity stimulated. All of the above possibilities are not mutually exclusive. Nevertheless, animals had, in essence, to learn a new discrimination. The fact that rats with CT transection display severe impairments in, but not complete elimination of, the expression of a depletion-induced sodium appetite suggests that the neurotomy does change something about the perceptual characteristics of sodium taste, but not completely (Breslin et al., 1993; Breslin et al, 1995; Markison et al., 1995; Frankmann et al, 1996).

The results from these behavioral experiments are helping to define the functional consequences of selective rewiring of gustatory nerves. As the story unfolds, it is becoming clear that some taste-related behaviors, such as unconditioned gaping to quinine, can recover from glossopharyngeal and chorda tympani nerve transection, if the posterior tongue taste buds are reinnervated, regardless of which lingual taste nerve is innervating them. For other taste related behaviors, such as the discrimination between NaCl and KCl, maintenance of normal function following transection of the lingual taste nerves requires that a specific nerve, the chorda tympani, reinnervate its native taste bud field, the anterior tongue. Thus, barring massive central reorganizational events, there appears to be a requirement for channeling appropriate peripheral signals through appropriate central gustatory circuits associated with input from the chorda tympani nerve. Although there remains much to learn about the underlying molecular and physiological events in the peripheral and central gustatory system associated with cross-regeneration of taste nerves, the behavioral outcomes provide an important context for guiding such an analysis and provide a functionally based interpretive framework for future experiments.

EXPERIMENTAL PROCEDURES

Subjects

Fifty-four male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) served as subjects in this experiment. Rats were individually housed in polycarbonate cages in a room where temperature and humidity were automatically controlled, and with a 12-hr light/dark cycle. Rats had *ad libitum* access to laboratory chow (Purina 5001; PMI Nutrition International, St. Louis, MO). Purified water (RO water, Millipore Elix 10, Bellerica, MA) was available *ad libitum* on the weekends. Water bottles were removed on Sunday afternoon and replaced Friday after the last testing session, and rats received their daily allotment of water during testing sessions Monday through Friday. Any rat whose body weight fell below 85% of its *ad libitum* weight was given 15 mL supplemental water. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Behavioral Procedures

Presurgical Training—Training and testing took place in a modified version of a previously described gustometer that had a left and right reinforcement spout instead of levers (Spector et al., 1990; St John et al., 1997). Rats responded by licking the reinforcement spout, and, if

correct, received water reinforcement. Taste solutions were delivered through a centrally positioned vertically oriented drinking spout. The animal was required to lick the dry drinking spout twice within 250 ms before fluid was delivered. This helped guarantee that the animal was engaged in licking when the stimulus was presented on a trial. After the drinking spout was initially filled each lick deposited approximately 5 μ L of fluid and licks were monitored by an electrical contact circuit passing less than 50 nA of current through the animal.

The rats were initially trained to lick water *ad libitum* from each spout (Spout Training). One session was devoted to each spout. Rats were then trained in the next 6 sessions (Side Training) to associate one reinforcement spout with 0.2 M NaCl (reagent grade chemicals; Fisher Scientific, Orlando, FL), and the other with 0.2 M KCl. Trials consisted of 5 lick samples from only a single stimulus in a given session during this phase of training. In the next training phase (Alternation), both 0.2 M NaCl and 0.2 M KCl were presented in a session and the rats were required to respond correctly to one stimulus a criterion number of times before the taste stimulus was switched. Correct responses were reinforced with access to water (10 s or 20 licks whichever came first) and incorrect responses were punished with a time-out during which the house lights were extinguished (see Table 1). This criterion number decreased systematically from 8 to 2. Finally, during Discrimination Training (I, II, and III), the stimuli were presented in randomized blocks, and the time-out increased while the allotted duration of the period to respond (i.e., limited hold) decreased. Also, the number of stimuli presented increased to six: 0.1 M, 0.2 M, and 0.4 M NaCl, and 0.1 M, 0.2 M, and 0.4 M KCl. Rats progressed through the phases of Discrimination Training once all animals had performed $\geq 85\%$ overall in a session during that phase.

Presurgical Testing—Testing began once all rats had passed the criterion performance for Discrimination Training III. Once a stimulus was presented, the rat was allowed 5 sample licks or 3 s, whichever came first. The limited hold was 5 s. If the rat responded correctly, it received up to 20 licks of water from the reinforcement spout, or 10 s, whichever came first. If the rat responded incorrectly or failed to respond, the rat received a 30-s time-out. The reinforcement or time-out was followed by a 6-s intertrial interval, during which the sample spout was rotated over a funnel and rinsed with purified water and evacuated by pressurized air. The sample spout then rotated back and the rat could initiate another trial. During the 40-min sessions, rats were able to initiate as many trials as possible. Presurgical testing consisted of six Control sessions (Monday, Wednesday and Friday sessions) in which stimuli were dissolved in purified water. There were also four Amiloride sessions (Tuesday and Thursday sessions), where 100 μ m amiloride hydrochloride served as the solvent. During these Amiloride sessions, all solutions including reinforcement water contained amiloride.

Postsurgical Testing—Postsurgical testing began 213–238 days after surgery to allow sufficient time for cross-regeneration to take place. During Postsurgical Testing weeks 1–4, rats were tested in 20 sessions under conditions identical to the Presurgical Control sessions. Postsurgical Testing weeks 5 and 6 were identical to Presurgical Testing, with six Control sessions and four Amiloride sessions. After the last testing session, a water control test was conducted in which all stimulus reservoirs were filled with water and half were arbitrarily designated as KCl and the other half were designated as NaCl, to determine whether performance was under taste stimulus control.

Surgery

Rats were placed into surgical groups balanced by overall presurgical performance, overall performance to each salt, body weight, 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). Supplemental doses were administered as necessary.

Table 2 provides a listing of the surgical groups and the status of the CT and GL upon postsurgical testing. The first 6 groups in this table represent controls for various degrees of lingual denervation and normal regeneration. Although the consequences of some of these manipulations on performance in this task are known, they were necessary to include to control for the very long duration that separated presurgical and postsurgical testing and to provide a source of comparison for the cross-regenerated surgical groups. The CT was exposed by retracting the ear canal and puncturing the tympanic membrane. The chorda tympani nerve was transected where it disappears behind the malleus on the posterior portion of the meatus. For groups with CT transection (CTx) and for the GL-AntTongue group, an additional surgery was performed 10–12 days prior to the start of postsurgical testing. The same approach was used, but during this procedure the ossicles were removed and the area was cauterized. This was done to guarantee that the CT had not regenerated. Rats in surgical groups in which the CT was encouraged to regenerate (CTr) or in which the CT was cross-wired to the posterior tongue (CT-PostTongue) did not receive this second surgery.

The GL was exposed by making a skin incision in the ventral neck and retracting the sublingual and submaxillary salivary glands and the sternohyoid, omohyoid, and posterior belly of the digastric muscles. The fascia under the hypoglossal nerve and near the external medial wall of the bulla was dissected. Approximately 10–12 mm of the GL was cut and removed (GLx). Rats in surgical groups in which the GL was encouraged to regenerate (GLr) had the nerve exposed and transected, but the two ends were left in an apposed position and a section was not removed. The incision was closed with sutures.

In both cross-regeneration surgeries, the GL was exposed as described above, but the hyoid was also retracted rostrally. To expose the CT, the connective tissue between the masseter and the digastric muscles was dissected. The pterygoid muscle and the anterior belly of the digastric muscle were retracted. A small transverse cut was made in the anterior portion of the pterygoid muscle to expose the CT near its junction with the lingual nerve proper. The nerve was carefully dissected away from the underlying sinus. In the CT-PostTongue surgery, the GL was transected close to its exit from the posterior lacerated foramen and routed under the mylohyoid muscle into the field of the central stump of the CT which was cut near where it joins the lingual nerve. An anastomosis was then formed between the ends of the two cut nerves using a single stitch of 11-0 monofilament suture (Ethicon, Inc., Somerville, NJ). In the GL-AntTongue surgery, the distal portion of the CT nerve was carefully stretched from the bulla, where it separated from the proximal end, and then threaded under the tendon of the digastric muscle. After the fascia was cleared, the CT was brought into the field of the central stump of the GL after the latter was transected close to the tongue and an anastomosis was formed as described. The skin incision was closed with nylon suture.

Penicillin G Procaine suspension (30,000 units sc) and ketorolac tromethamine (2 mg/kg body mass sc) were administered for 3 days following surgery. Rats were given a supplemental diet of wet mash (powdered 5001 chow with purified water) mixed with a high-calorie dietary supplement (Nutrical; Evsco Pharmaceuticals, Buena, NJ). They also received 40–45 mL diluted sweetened condensed milk (1:1 Carnation sweetened condensed milk to water with 1 mL Polyvisol multivitamin supplemental drops, Enfamil, Eversville, IN). This diet was continued until the rats had recovered from surgery. Rats were housed individually in hanging wire mesh cages until they had returned to their presurgical body weight and their incisions had healed. One animal died during surgery and 3 animals were euthanized due to illness before postsurgical testing began.

Histology

The rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with saline followed by 10% buffered formalin. The tongue of each rat was removed and stored in

10% buffered formalin. The anterior portion of the tongue from the intermolar eminence to the tip was placed in purified water for 10 minutes, dipped in 0.5% methylene blue until well saturated (less than 1 minute) and rinsed with purified water. The epithelium was removed from the underlying muscle and connective tissue, pressed between two slides, and the total number of fungiform papillae and taste pores were counted under a light microscope. The circumvallate and foliate papillae were embedded in paraffin and cut into 10- μ m sections. These sections were mounted on slides, stained with hematoxylin and eosin, and taste buds were counted under a light microscope. All tissues were coded so that the surgical condition was unknown to the counters.

Data Analysis

The number of fungiform papillae, taste pores, percentage of fungiform papillae with taste pores, and total number of intact circumvallate taste buds were analyzed with one-way analyses of variance (ANOVAs). The overall percentage correct on trials with a response was calculated for each animal. Group averages were calculated and compared using ANOVAs. When an overall ANOVA indicated the presence of differences, we conducted paired comparisons between surgical groups using a procedure that maintained the False Discovery Rate at $p=.05$ (Benjamini and Hochberg, 1995). Individual performance during the water control test was tested for positive differences from chance with the one-tailed normal approximation of the binomial distribution. The conventional $p \leq .05$ was used as the criterion for statistical significance (i.e., α). Fifteen rats were removed from the experiment due to either technical problems encountered with one of the gustometers during the first week of postsurgical during testing ($n=7$) or because of inappropriate nerve regeneration or failure thereof based on the histology ($n=8$). Data collected from these rats are not included in any analysis. Moreover, because the CTr (unil) + CTx + GLX group was left with only two rats after the removal of the rats described above, this group's data was not included.

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Figure 1.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials on the last 6 presurgical test sessions without amiloride treatment. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. It is clear that all of the groups were displaying high levels of performance in the task before surgery. SHAM: Sham-operated controls; GLx: permanent bilateral transection of the glossopharyngeal nerve; CTr+GLx/GLr: bilateral regeneration of the chorda tympani nerve coupled with either bilateral regeneration of the glossopharyngeal nerve or permanent bilateral transection of the glossopharyngeal nerve; CTx: permanent bilateral transection of the chorda tympani nerve; CTx+GLr+GLx: unilateral regeneration of the glossopharyngeal nerve coupled with contralateral permanent transection of the glossopharyngeal nerve and permanent bilateral transection of the chorda tympani nerve; CT-PostTongue: bilateral cross anastomoses of the central portion of the chorda tympani nerve with the peripheral portion of the glossopharyngeal nerve resulting in bilateral reinnervation of the posterior tongue taste buds by the chorda tympani nerve coupled with lack of taste nerve innervation of the anterior tongue; GL-AntTongue: bilateral cross anastomoses of the central portion of the glossopharyngeal nerve with the peripheral portion of the chorda tympani nerve resulting in bilateral reinnervation of the anterior tongue taste buds by the glossopharyngeal nerve coupled with lack of taste nerve innervation of the posterior tongue.

Figure 2.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials on the 4 presurgical test sessions with 100 μ M amiloride hydrochloride serving as the solvent. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. Amiloride treatment severely disrupted the NaCl vs. KCl discrimination presurgically. See Figure 1 caption for definition of group abbreviations.

Postsurgical Performance: Week 1

Figure 3.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials on the first 5 postsurgical surgical test sessions. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. Transection of the GL alone had no effect on performance. Transection of the CT severely impaired performance which recovered if the CT regenerated into its native taste bud field in the anterior tongue. If the CT regenerated into the posterior tongue leaving the anterior tongue absent of taste innervation or if the GL regenerated into the anterior tongue leaving the posterior tongue denervated, performance was no better than the CTx group. In other words, neither cross-regeneration condition led to recovery of function. See Figure 1 caption for definition of group abbreviations.

Figure 4.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials on last 6 test sessions without amiloride treatment during weeks 5 and 6 of postsurgical testing. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. Some rats in all groups that were impaired during the earlier sessions of postsurgical testing improved their performance over the 6 weeks of postsurgical testing suggesting that they were able to “relearn” the discrimination based on the input remaining in the intact gustatory nerves. See Figure 1 caption for definition of group abbreviations.

Figure 5.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials for weeks 1–4 of testing

Figure 6.

The mean ($\pm$ se) overall proportion correct on the NaCl vs. KCl discrimination task collapsed across all NaCl and KCl trials on the 4 test sessions for which 100 μ M amiloride hydrochloride served as the solvent during weeks 5 and 6 of postsurgical testing. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. The performance in all rats was severely disrupted by amiloride treatment. See Figure 1 caption for definition of group abbreviations.

Figure 7.

The mean ($\pm$ se) total number of taste pores in the fungiform papillae (left panel), taste buds in the circumvallate papillae (middle panel), and taste buds in the foliate papillae (right panel) for animals included in the behavioral analysis. The different symbols represent the performance of individual rats and are consistent within a specified group across all figures. Counting was conducted blindly. See Figure 1 caption for definition of group abbreviations.

Figure 8.

Hypothesized interpretation for the effects of cross-regeneration of the chorda tympani (CT) and glossopharyngeal (GL) nerves on discrimination performance. A. In the intact case NaCl selectively stimulates the amiloride-sensitive channels on taste receptor cells found in the anterior tongue (Ant.) as well stimulating less cation-selective amiloride-insensitive channel (s) on taste receptor cells in both the anterior and posterior tongue (Post.). Receptors on the palate,, some of which are amiloride-sensitive, supplied by the greater superficial petrosal (GSP) nerve also contribute to normal salt discrimination. The cation specificity of the amiloride-sensitive GSP fibers remains unknown, however, and it is assumed here to be similar to the anterior tongue. Growing evidence suggests that branches of the facial nerve (i.e., CT and GSP) including the chorda tympani provide necessary and sufficient input to neural circuits subserving qualitative taste discrimination. Thus, the GL provides irrelevant information for this particular task. B. When the CT cross-reinnervates the posterior tongue in the absence of the GL, there is no longer a discriminable signal arising from the taste receptor cells of the anterior tongue that unequivocally distinguishes between NaCl and KCl. Input is still provided

from the palate by the intact GSP, but the brain is now receiving changed total peripheral signals leading to some change in salt perception. C. When the GL cross-reinnervates the anterior tongue in the absence of the CT, although a discriminable signal is available from the taste receptor cells it is channeled through neural circuits that do not contribute to discriminative function. Although the GSP still provides some discriminable signal, the circuits involved in qualitative taste discrimination are now receiving a changed peripheral signal leading to changed salt perception. The framework (B & C) shown here assumes that phenotype of the regenerated taste receptor cells is maintained in the face of reinnervation from a different gustatory nerve. Moreover, it is assumed that there is not massive reorganization of central circuits. The superior laryngeal branch of vagus which innervates taste buds in the laryngeal epithelium thought to be primarily involved with protection of the airways is not shown. The presence of an intact GSP is likely responsible for keeping performance above chance when the CT is transected and serves as the basis for the recovery of function with extended postsurgical testing as animals learn a new taste discrimination based on the changes in salt perception which occurred as a function of the changes in peripheral input. The perceptual changes need not be qualitative per se but could also involve decreases in intensity, sensation rise and decay times, or simply changes in the oral locus of the signal.

Table 1

Training parameters.

Phase	# of Sessions	LH (s)	TO (s)	Stimuli	Stimulus Presentation
Training					
Spout	3	N/A	N/A	H ₂ O	Constant
Side	6	180	0	0.2M NaCl or KCl	Constant
Alternation	4	15	10	0.2M NaCl and KCl	Criterion ^a
Discrimination I	3–4	10	10	0.2M NaCl and KCl	Semi-random ^b
Discrimination II	3–5	10	20	0.1, 0.2, and 0.4 M NaCl and KCl	Semi-random
Discrimination III	3–4	5	30	0.1, 0.2, and 0.4 M NaCl and KCl	Semi-random
Presurgical Testing	10	5	30	0.1, 0.2, and 0.4 M NaCl and KCl [with or without 100µM amiloride]	Semi-random
Postsurgical Testing	30	5	30	0.1, 0.2, and 0.4 M NaCl and KCl [with or without 100µM amiloride]	Semi-random

Note: LH = limited hold, the amount of time (in seconds) the rat has to respond after sampling the stimulus. TO = time out (in seconds) for incorrect responses.

^a the alternation criteria used: 8, 6, 4, 2.

^b Stimuli were presented in randomized blocks of 6 without replacement.

Table 2Description of Surgical Groups^{1,2}

Surgical Group	Status of Chorda Tympani Nerve (CT)	Status of Glossopharyngeal Nerve (GL)	Final Group Size³
SHAM	Intact	Intact	N=4
CTx	Transected	Intact	N=5
GLx	Intact	Transected	N=4
CTr + GLr ⁴	Regenerated to anterior tongue	Regenerated to posterior tongue	N=3
CTr + GLx ⁴	Regenerated to anterior tongue	Transected	N=4
CTx + GLr (unil) + GLx	Transected	Bilaterally transected, unilaterally regenerated to the posterior tongue	N=4
CT-PostTongue	Regenerated to posterior tongue	Transected	N=4
GL-AntTongue	Transected	Regenerated to anterior tongue	N=5

¹ All transections are bilateral unless otherwise noted.

² Six rats received a CTx + CTr (unil) + GLx surgery, but only two animals were histologically confirmed so those animals were not included in the paper.

³ Some animals were discarded due to problems that occurred during testing or because histological analysis indicated inappropriate regeneration or lack of regeneration.

⁴ The CTr+GLr group was combined with the CTr+GLx group for statistical analysis to increase power because there was no obvious difference in the postsurgical behavior of these rats.