Experimentally cross-wired lingual taste nerves can restore normal unconditioned gaping behavior in response to quinine stimulation

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King CT, Garcea M, Stolzenberg DS, Spector AC. Experimentally cross-wired lingual taste nerves can restore normal unconditioned gaping behavior in response to quinine stimulation. Am J Physiol Regul Integr Comp Physiol 294: R738–R747, 2008. First published January 9, 2008; doi:10.1152/ajpregu.00668.2007.—Studies examining the effects of transection and regeneration of the glossopharyngeal (GL) and chorda tympani (CT) nerves on various taste-elicited behaviors in rats have demonstrated that the GL (but not the CT) nerve is essential for the maintenance of both an unconditioned protective reflex (gaping) and the neural activity observed in central gustatory structures in response to lingual application of a bitter substance. An unresolved issue, however, is whether recovery depends more on the taste nerve and the central circuits that it supplies and/or on the tongue receptor cell field being innervated. To address this question, we experimentally cross-wired these taste nerves, which, remarkably, can regenerate into parts of the tongue they normally do not innervate. We report that quinine-stimulated gaping behavior was fully restored, and neuronal activity, as assessed by Fos immunohistochemistry in the nucleus of the solitary tract and the parabrachial nucleus, was partially restored only if the posterior tongue (PT) taste receptor cell field was reinnervated; the particular taste nerve supplying the input was inconsequential to the recovery of function. Thus, PT taste receptor cells appear to play a privileged role in triggering unconditioned gaping to bitter tasting stimuli, regardless of which lingual gustatory nerve innervates them. Our findings demonstrate that even when a lingual gustatory nerve (the CT) forms connections with taste cells in a non-native receptor field (the PT), unconditioned taste rejection reflexes to quinine can be maintained. These findings underscore the extraordinary ability of the gustatory system to adapt to peripherally reorganized input for particular behaviors.

oromotor reflex; regeneration; bitter taste; nerve injury

THE GUSTATORY SYSTEM HAS BEEN described as an ideal model to examine the role of degenerative and regenerative processes on sensory function due to its inherent and notable plasticity (e.g., 25). For example, taste receptor cells normally turnover about every 10 days (5, 11), yet somehow, in the midst of continual synaptic rearrangement with first-order gustatory neurons, the replaced cells seemingly sustain normal afferent messages. Moreover, when lingual taste nerves are cut, taste buds in the denervated oral region degenerate, either partially or completely, depending on the species, and the transected fibers regenerate to reinnervate their native receptor field (e.g., 8, 9, 12, 13, 19, 23, 39, 55, 58, 60). Following reinnervation of the epithelium by the nerve fibers, the taste buds return and in the rodents examined, behavioral function is restored (e.g., 3, 4, 6, 14, 27, 29, 30, 45). Such plasticity is indeed remarkable, but it is unclear whether the recovered function is dependent upon the tongue receptor field being reinnervated (the source of the input) or upon the nerve and the central circuits it supplies, or both.

Oakley (36) was the first to address this question in a fascinating and innovative set of experiments using rats. He transected the chorda tympani nerve (CT), which normally innervates the anterior tongue (AT) taste buds, and formed a cross-anastomosis with the transected glossopharyngeal nerve (GL) such that the regenerating CT fibers reinnervated the taste buds of the posterior tongue (PT), the normal target of the GL. In other rats, the converse type of cross-anastomosis was formed. Using whole-nerve electrophysiology, he showed that it was the characteristic chemical sensitivity of the taste receptor cells of the region of the tongue reinnervated that dictated the response properties of the regenerated nerve, not the other way around. For example, the intact rat GL, which normally responds well both to quinine, a bitter-tasting alkaloid, and sodium saccharin, an artificial sweetener, and modestly to NaCl placed on its receptor field, responded well to NaCl and relatively poorly to quinine and saccharin when it cross-reinnervated the AT taste buds. Likewise, when the CT nerve cross-reinnervated the PT, it adopted the response profile of an intact GL and uncharacteristically responded well to quinine and saccharin and poorly to NaCl (36). Nejad and Beidler (34) reached a similar conclusion when they cross-wired the CT and the greater superficial petrosal nerve, which innervates taste buds in the palate. In contrast, Ninomiya (35), who examined single-fiber electrophysiological responses of cross-regenerated gustatory nerves in the mouse, found that the inhibitory effectiveness of amiloride (which blocks epithelial sodium channels thought to be involved with sodium taste transduction) in producing responses to NaCl was dependent on the cross-regenerated nerve, not the receptor field it was innervating.

Of course, restoration of responsiveness in a cross-regenerated nerve to chemical stimulation of the tongue does not guarantee that the sensory signals will be properly interpreted by the brain and support normal taste-related behavior. Indeed, the afferent projections of the CT and GL nerves terminate in relatively segregated regions (but with some overlap) in the gustatory nucleus of the solitary tract (gNST), the first central relay in the gustatory system (22, 31, 33); and thus, in these cross-wired animals, afferent signals may have been channeled inappropriately into central brain circuits. In a later experiment, the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Oakley (37) did provide a crude behavioral test of taste function in rats that had the GL routed to the AT on one side and all of the remaining lingual gustatory nerves transected. These animals displayed relatively normal preference-aversion functions for sucrose, saccharin, and quinine, as assessed in 48-h two-bottle tests. The significance of such behavioral results is undermined by the fact that the two-bottle preference test can be influenced by nontaste factors and is not a particularly sensitive measure of gustatory competence (see Ref. 41). In some cases, complete gustatory nerve deafferentation of the tongue produces minor, if any, alterations in taste solution preference or aversion measured in this way (e.g., 2, 17, 40, 54).

In order for a test of the functional consequences of gustatory nerve cross-regeneration to have meaning, the taste-related behavior measured must be unequivocally disrupted by transection of one lingual taste nerve but not the other. Moreover, the impaired behavior must recover upon normal regeneration of the transected nerve into its native receptor field. These experimental requirements are satisfied through the measurement of the gape (see Fig. 1 inset), a hallmark oromotor rejection reflex unconditionally displayed by rats and other mammals in response to bitter solutions. Transection of the GL severely blunts quinine-stimulated gaping in rats, whereas transection of the CT has much less of an effect (18, 27, 49). Transection of the GL also eliminates the characteristic pattern of quinine-stimulated neuronal Fos expression, which is a proxy for neural activation, in hindbrain centers involved in gustatory processing, in particular, the medial dorsal subfield (MD) of the gNST (24, 27, 28, 51) and the waist area of the parabrachial nucleus (PBN; 26). Again, transection of the CT has only marginal effects (28). Importantly, when the GL regenerates into the PT, these functional measures return to normal values (26, 27).

The necessity of the GL and its primary central targets, per se, in sustaining functional rejection responses to bitter-tasting substances is disputable, however, because taste bud cells located in the PT prominently express receptors that bind with bitter-tasting ligands (called T2Rs), the AT only sparingly expresses them (1). This difference suggests that a weaker or different “bitter” signal arises from the AT. Even so, the CT can sufficiently mediate other quinine-stimulated taste-related behaviors. For example, in the absence of the GL, rats are still able to detect and discriminate quinine (46, 47); to display quinine-concentration-dependent suppression of licking in brief-access tests (44), albeit sometimes in a slightly less sensitive manner (32); and even to gape in response to sucrose conditioned to be aversive (10). Collectively, these findings have led to the suggestion that the input of the GL and its associated central circuits play a privileged role in triggering gapes to unconditioned taste stimuli.

Here, we rewired the adult gustatory system as Oakley (36) did to assess for the first time whether quinine-stimulated gaping, a reliable, stereotypical oromotor rejection response, and the associated neuronal activation, as assessed by Fos immunohistochemistry, in two critical brain stem regions, the gNST and PBN, depends more on the receptor field origin of the taste input or on the nerve that channels the signal into the brain.

MATERIALS AND METHODS

Subjects

Forty-seven male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) weighing ~400 g at the time of nerve surgery were individually housed in polycarbonate tub cages. After surgery, they were placed in hanging wire-mesh cages until the incision site healed. Laboratory chow (5001; Purina Mills, Inc. St. Louis, MO) and water were available ad libitum except where noted below. The temperature, humidity, and light cycle (12:12-h light-dark cycle) were automatically controlled. All experimental protocols were approved by the Institutional Animal Care and Use Committees of the University of Florida and Stetson University.

Surgical Procedures

All surgeries were conducted in rats that were deeply anesthetized (125 mg/kg body mass ketamine hydrochloride + 5 mg/kg body mass xylazine im; supplemental doses delivered as necessary). The rats received subcutaneous injections of penicillin (30,000 units) and ketorolac tromethamine (2 mg/kg body mass) for 3 days following surgery. Wet mash (powdered chow mixed with water and supplemented with a calorically dense suspension, Nutri-Cal, Evsco Pharmaceuticals, Buena, NJ) was available ad libitum in addition to a milk diet (1 part sweetened condensed milk in 2 parts water) for 3 days. In some cases, the wet mash was continued until body mass displayed signs of steady increases.

Four types of surgical manipulations of the lingual gustatory nerves were performed bilaterally to allow for reinnervation of either the PT...
taste receptor cells or the AT taste receptor cells. In addition, one surgery, designed to prevent the reinnervation of both taste receptor cell fields, was performed. Two sham-surgical groups (one stimulated with quinine, the other stimulated with water) were also included, for a total of seven groups. All nerve surgeries were conducted 149–231 days before behavioral testing to allow time for successful regeneration of the nerves. The sample sizes listed below under Cross-anastomosis and Control nerve surgeries indicate the original number of animals undergoing surgery in that group. Table 1 lists the total number of animals analyzed per group and brain region. Animals were discarded when necessary, for example, when brain sections were damaged or when, as assessed by histological analysis of the tongue tissue (see below), successful bilateral reinnervation of the tongue was not observed or significant unintended regeneration occurred.

Cross-anastomosis. The cross-anastomosis procedures were modified from those described by Oakley (38) and performed bilaterally. The CT was exposed near its junction with the lingual nerve proper by retracting the pterygoid muscle up and laterally, and retracting the transverse ramus muscle rostrally. A small transverse cut was made in the anterior portion of the pterygoid muscle to reveal the lingual nerve and the CT. The GL was exposed along the external medial wall of the bulla and was followed distally for about several millimeters after retracting the posterior belly of the digastric muscle, the omohyoid muscle, and the sternohyoid muscle. The hyoid was retracted rostrally. In one group (GL→AT; n = 10), the cross-anastomosis between the central portion of the GL and the peripheral portion of the CT was achieved by first gently pulling the CT from the bulla until it separated from the proximal end and then trimming the distal end and carefully dissecting it from the fascia. The GL was then exposed and stretched from the tongue and cut, and the peripheral CT was threaded under the tendon of the digastric muscle and placed in the field of the GL. The ends of the two nerves were then carefully joined with 11-0 monofilament suture. In another group (CT→PT; n = 8), the cross-anastomosis between the central portion of the CT and the peripheral portion of the GL was achieved by cutting the GL distal to the tongue close to its exit from the posterior lacerated foramen and carefully stretching the GL, under the mylohyoid muscle, into the area of the central stump of the CT, which was transected close to where it joins the lingual nerve proper. The ends of the two nerves were then sutured as described above.

Control nerve surgeries. To control for the effects of “regeneration” per se, some animals had their nerves transected in a manner that allowed for normal reinnervation of either the AT or PT receptor fields. In these animals, both nerves were exposed as described above, but in one group of animals (CT→AT; n = 6), the CT was simply cut, and the two ends were left apposed. In this way, regenerating CT fibers would innervate their normal targets in the AT. In these same animals, the GL was transected in a way that prevented its regeneration (removing ~8 mm of the nerve), so that only the AT would be reinnervated by its native nerve. In another control group (GL→PT; n = 5), the GL was simply cut and the two ends were left apposed to promote normal reinnervation of the PT, and the CT was transected in a way that discouraged its regeneration into the AT (removing ~8 mm of the nerve).

A surgical control group included rats for which both gustatory nerves were simply exposed. Some of these rats received quinine as the intraoral stimulus on “test” day (SHAM-Q; n = 5) and some received dH2O (SHAM-W; n = 6).

A final group included rats that underwent a bilateral double-nerve transection (NoREG, n = 4), in which portions (at least 8–10 mm) of both gustatory nerves were removed to prevent both the regeneration of the taste nerves and the reinnervation of their receptor fields on the tongue.

Intraoral cannula surgeries. The intraoral cannulas through which taste stimuli could be directly infused into the mouth (16, 28) were bilaterally implanted in deeply anesthetized rats 2 wk before the commencement of behavioral procedures for each rat. During this surgery, the CT, where it passes through the middle ear, was cut in three groups (GL→AT, GL→PT, and NoREG rats) to ensure that any taste buds histologically observed 2 wk later were not due to reinnervation by the CT into the AT. The comparable surgery (cutting the GL in the CT→PT, CT→AT, or NoREG rats was not performed because in preliminary work, there was no observable nerve tissue to cut, and we did not want to risk losing these valuable animals. We are confident, however, that the native GL did not regenerate into the PT in the CT→PT group because the pattern of neural activity observed in the gNST did not mirror that typically found for a normally regenerated GL nerve (see Figs. 3–5). All rats received subcutaneous injections of penicillin (30,000 units) and ketorolac tromethamine (2 mg/kg body mass) for 3 days following the implantation of the intraoral cannulas, and additional injections were given as needed throughout the recovery period. Wet mash was available ad libitum to all rats throughout the recovery period, which lasted for 14 days. The cannulas were cleaned daily to maintain patency and prevent infection.

Stimulus Delivery

The behavioral procedures were based on those described previously (28). For three habituation days, the procedures emulated exactly those described for the test day, except that all animals received distilled water (dH2O), as the intraoral stimulus on these habituation days. On the test day, the animal’s left cannula was

| Table 1. Summary of subjects per nerve condition used for analysis |
|---|---|---|---|
| Abbreviated in Text as | Bilateral Surgery | NST | PBN |
| Controls | | | |
| SHAM-Q | No nerve transection (quinine-stimulated) | n = 5 | n = 5 |
| SHAM-W | No nerve transection (water-stimulated) | n = 6 | n = 6 |
| Reinnervation of only the posterior tongue | | | |
| GL→PT | GL sutured to GL (+ CT transected) | n = 5 | n = 4 |
| *CT→PT | CT sutured to GL | n = 5 | n = 5 |
| No reinnervation | Both GL and CT transected | n = 4 | n = 3 |
| NoREG | | | |
| Reinnervation of only the anterior tongue | | | |
| CT→AT | CT sutured to CT (+ GL transected) | n = 4 | n = 4 |
| *GL→AT | GL sutured to CT | n = 7 | n = 7 |

SHAM-Q, rats that received quinine as the intraoral stimulus; SHAM-W, control group that received distilled water; GL, glossopharyngeal nerve; PT, posterior tongue; CT, chorda tympani nerve; NoREG, rats that underwent a bilateral double nerve transection; AT, anterior tongue; NST, nucleus of the solitary tract; PBN, parabrachial nucleus. *Denotes cross-wired group.
attached via polyethylene tubing to a syringe on an infusion pump (Harvard Apparatus, South Natick, MA), and the animal was placed in a cylindrical Plexiglas chamber for a 1-h adaptation period. For the next 30 min, 7 ml of either dH2O or 3 mM quinine-hydrochloride were infused through the cannula at a rate of 0.233 ml/min. During the first minute, an experimenter was present in the room to videotape the rat for subsequent scoring of taste reactivity behaviors. Responses were recorded using S-VHS equipment (Panasonic AW-E300 convertible camera and SLV-R1000 video cassette recorder). At the end of the 30th min, the infusion pump was turned off, but the animal was left in the behavioral arena for 45 min before either being returned to its home cage (habituation days) or anesthetized for perfusion (test day).

Brain and Tongue Histology

Immediately after the 45-min postinfusion period on the test day, the rats were deeply anesthetized with an overdose of pentobarbital sodium (80 mg/kg body mass) and perfused intracardially with chilled, heparinized 0.15 M NaCl, followed by sodium phosphate-buffered 4% paraformaldehyde. Following an overnight postfixation at 4°C, the brains were cut in the coronal plane (75 μm) using a Vibratome. Every other section was processed for the Fos protein as follows: the sections were pretreated for 20 min with sodium borohydride (1% in potassium phosphate-buffered saline, KPBS), rinsed in KPBS, and then incubated in rabbit polyclonal antibody [c-Fos (4): sc-52, Santa Cruz Biotechnology, Santa Cruz, CA] at a dilution of 1:10,000 in 0.4% Triton X-100 in KPBS for 72 h at 4°C. After several rinses in KPBS, the sections were placed in biotinylated goat anti-rabbit IgG (Zymed, San Francisco, CA) at a dilution of 1:600 for 4 h at room temperature. Following several rinses in KPBS, the sections were placed in an avidin-biotinylated peroxidase complex (ABC kit: Vector Laboratores, Burlingame, CA) overnight at 4°C and again rinsed in KPBS. The sections were then placed in sodium phosphate buffer containing 0.03% diaminobenzidine, 0.008% nickel ammonium sulfate, and 0.0075% hydrogen peroxide. Finally, the reacted sections were mounted on chrome-alum subbed slides, dehydrated, and coverslipped. Alternate sections were mounted and stained with 0.1% thionin to aid in delineating the borders of the gNST and PBN.

The tongues were removed and postfixed in 10% buffered formalin for several weeks. The AT of each animal was isolated, stained with 0.5% methylene blue, rinsed with distilled water to remove excess stain, and flattened after the underlying muscle and connective tissue were removed. The sheet of tissue was then pressed between two glass slides for taste bud counting with light microscopy. Portions of the PT containing the circumvallate papilla on the dorsal midline and the foliate papillae on the lateral aspects were removed, embedded in paraffin, sectioned at 10 μm, and stained with hematoxylin and eosin.

Microscopic analysis of brain and tongue tissues. The tagging of Fos-positive cells in the NST and PBN was performed by experimenters who were naïve regarding the group to which the rats were assigned. Brain sections were observed under ×4 to ×40 objectives with a Zeiss (Oberkochen, Germany) Axioscope microscope equipped with a video camera coupled to a video monitor and computer. Video images were captured using Zeiss Image Software.

Five standard sections of the NST spaced throughout its rostrocaudal extent were selected for analysis (see Ref. 28). Four of these sections (approximately equidistant from each other) were selected from the gNST (rostral to the area postrema). These gNST sections were termed rostral (RgNST), intermediate rostral (IRgNST), intermediate caudal (ICgNST), and caudal (CgNST). The one section taken at the level of the area postrema was considered nongustatory. All Fos-positive nuclei within the borders of the NST in each of these sections were individually identified and electronically “tagged” for later counting. A neuron was considered Fos-positive if its nucleus was completely stained whether the staining was dark, medium, or light. The “tagged” sections were printed alongside their adjacent Nissl-stained sections. Using the thionin-stained tissue as a guide, the gNST was parceled into 6 “subfields” based on its medial-lateral and dorsal-ventral dimensions (28, 51; see also Refs. 21, 22, 57), and the number of Fos-labeled neurons in each subfield was tallied.

Sixteen 75-μm sections from the caudal two-thirds of the PBN from each subject were also used in this study. Every other of these sections was Nissl stained, and the alternate sections were processed for the Fos protein. The section in which Nissl-stained cell bodies were first apparent within the central medial subdivision was identified as the most caudal PBN section for analysis in each subject. The most rostral of these sections (the 16th section) was roughly at the level at which the brachium abuts the mesencephalic trigeminal tract.
For each of the eight Fos immunohistochemically stained sections, all Fos-positive neurons within the PBN borders were electronically tagged for later counting as described above. The Nissl-stained sections, which were printed out alongside the Fos-labeled sections, were used to help delineate the subdivisions of the PBN.

The counting of fungiform, circumvallate, and foliate taste bud pores was also performed by an experimenter naïve to the experimental assignment of the subjects. The number of intact taste pores stained with methylene blue on the surface of the AT relates well to the number of morphologically intact taste buds in the fungiform papillae (45), and so it was used as an indicator of reinnervation. In the case of the circumvallate and foliate papillae, taste pores were also counted. Sometimes, a taste pore was not captured by the sectioning, but there was clear evidence of convergence of the apical membranes of taste bud cells, and these were counted as normal.

Behavioral Analysis

One experimenter unaware of the group assignment of the subjects viewed, in slow motion and/or frame-by-frame, the videotaped responses for the 1st min of the infusion period. On the test day, a variety of oromotor taste reactivity behaviors, including both aversive and ingestive domains were scored (42), but the gape, a hallmark oromotor rejection response, was chosen for analysis because of its unequivocal form and its relevance to prior research. On the last water habituation day preceding the test day, only gapes were scored. Every occurrence of a gape was noted and summed for the 1-min epoch.

Statistical Procedures

Separate one-way ANOVAs, one for each dependent variable, were conducted using the seven groups to assess main effects. If the ANOVA revealed significant differences, then post hoc Bonferroni tests were performed. The statistical rejection criterion used for significance was $P \leq 0.05$.

RESULTS

Gaping Behavior

The results unequivocally indicated that gaping triggered by oral stimulation with quinine was contingent upon the receptor field origin of the taste input rather than on the nerve supplying signals to central targets (Fig. 1; $F_{6,29} = 26.98, P < 0.00001$). After complete bilateral transection of the lingual gustatory nerves, reinnervation of the PT taste buds alone, either by the native GL nerve (GL→PT) or the cross-regenerated CT nerve (CT→PT), resulted in virtually as many gapes to quinine as found in sham-operated rats (SHAM-Q; post hoc tests, $P = 1.00$). On the contrary, reinnervation of the AT receptor field alone, either by the native CT nerve (CT→AT) or the cross-regenerated GL nerve (GL→AT) produced a striking attenuation of gapes compared with sham-operated rats (post hoc tests, $P < 0.0001$). Indeed, the quinine-stimulated gaping behavior of the GL→AT rats emulated the behavior elicited by water in intact rats (SHAM-W; post hoc test, $P = 1.00$), confirming that the GL was not capable of supporting normal unconditioned gaping in response to quinine stimulation when the nerve reinnervated its non-native receptor field. Importantly, the number of gapes in response to water on the final habituation day was near zero (Fig. 1B), and there were no differences between the groups ($F_{6,29} = 1.42, P = .242$). Thus, the effects of the nerve surgery conditions on gaping to quinine cannot be attributed to the mechanical or thermal features of the stimulus. Histological analysis of taste buds was used to confirm nerve regeneration (Fig. 2).

gNST. Figs. 3 and 4 depict the quinine-stimulated Fos response within the MD of the gNST, a gustatory subfield that occupies the medial dorsal 1/6 of the gNST along its rostrocaudal extent (see Fig. 5, inset) and typically displays intense Fos expression following intraoral quinine stimulation that quantitatively and characteristically differs from that observed following water stimulation (24, 28, 51). In the current study, similar results were realized for both SHAM-Q rats and rats with reinnervation of the PT by its native nerve (GL→PT). In these two groups, quinine-stimulated Fos expression in MD in the IRgNST ($F_{6,29} = 31.179, P < 0.0001$), IRgNST ($F_{6,29} = 36.274, P < 0.0001$) and ICgNST ($F_{6,29} = 20.894, P < 0.0001$) was comparable (post hoc tests, $P = 1.00$) and significantly higher than the Fos expression in water-stimulated controls (post hoc tests, $P < 0.0001$). On the other hand, in rats with cross-wired PTs (CT→PT), the numbers of quinine-stimulated Fos neurons were comparable to those in SHAM-Q and GL→PT rats but only within the rostral-most section (RgNST) of the nucleus (Fig. 3A; post hoc tests, $P = 1.00$). At more caudal levels of the nucleus, in IRgNST and ICgNST, quinine-stimulated Fos expression decreased in the rats with cross-wired PTs (CT→PT vs. SHAM-Q, $P < 0.02$; CT→PT vs. GL→PT, $P < 0.0004$; Fig. 3, B and C), despite that all three groups gaped at roughly equivalent rates. In fact, within ICgNST, the quinine-stimulated Fos response was different from the response observed following water stimulation when the nerve reinnervated its non-native receptor field. Importantly, the number of gapes in response to water on the final habituation day was near zero (Fig. 1B), and there were no differences between the groups ($F_{6,29} = 1.42, P = .242$). Thus, the effects of the nerve surgery conditions on gaping to quinine cannot be attributed to the mechanical or thermal features of the stimulus. Histological analysis of taste buds was used to confirm nerve regeneration (Fig. 2).
stimulation \((P = 0.467)\). Thus, reinnervation of the PT by the non-native CT nerve only partially restored the characteristic quinine-stimulated Fos-pattern (Fig. 5) that is typically observed in the gNST following intraoral application of quinine \((24, 28, 51)\).

In groups with only reinnervation of the front of the tongue (e.g., GL→AT and CT→AT rats), quinine-stimulated neural activity in MD was significantly lower than that observed in SHAM-Q rats within the RgNST (post hoc tests, \(P < 0.0001\)), the IRgNST (post hoc tests, \(P < 0.0001\)), and the ICgNST (post hoc tests, \(P < 0.0001\)). Moreover, the Fos response in these quinine-stimulated animals was remarkably similar to the expression pattern (Fig. 5) observed throughout the nucleus in water-stimulated controls (SHAM-W, post hoc tests, \(P = 1.00\)).

PBN. As has been reported previously \((26, 50, 61, 62)\), many Fos-positive neurons were observed in the taste-responsive waist region of the PBN in sham-operated animals following intraoral stimulation with quinine but not with distilled water \((F_{6,27} = 12.150; P < 0.0001; \text{post hoc test, } P = .0008\); Figs. 6 and 7). As with MD in the RgNST, reinnervation of the PT by either the native GL nerve (GL→PT) or the non-native CT nerve (CT→PT) restored the numbers of Fos-positive quinine-stimulated neurons to normal values \((\text{post hoc tests, } P = 1.00\) compared with SHAM-Q), while reinnervation of the AT by either nerve (CT→AT or GL→AT) did not \((\text{post hoc tests, } P < 0.006\) compared with SHAM-Q). In fact, quinine stimulation in these AT-reinnervated animals yielded numbers that were similar to those obtained in water-stimulated controls \((\text{post hoc tests, } P = 1.00)\).

In both the external lateral \((F_{6,27} = 4.892, P < 0.002)\) and external medial \((F_{6,27} = 3.391, P < 0.013)\) subdivisions of the PBN, areas that have also been implicated in gustatory processing \((20, 26, 50, 62)\), more Fos-positive neurons were found in sham-operated rats following stimulation with quinine compared with water \((\text{post hoc tests, } P < 0.035\); Figs. 6 and 7). The mean numbers of Fos-positive neurons in these external subdivisions across the nerve-manipulated groups appeared to follow a similar pattern observed for the waist area \((\text{e.g., normal quinine-stimulated Fos response with PT reinnervation but not with AT reinnervation})\), but the comparisons failed to reach statistical significance.

**DISCUSSION**

**Cross-regeneration effects.** The main purpose of this investigation was to assess whether quinine-stimulated gaping and the associated neural activity in brain stem gustatory nuclei depended more on the receptor field origin of the taste input or on the nerve that channels the signal into the brain or both. Because either taste nerve was clearly able to support normal quinine-stimulated gaping behavior when routed into the PT but not able to do so when routed into the AT, the specific nerve that channels the signal appears to play a negligible role. Instead, the data point toward the tongue receptor cell field as the critical factor. A straightforward explanation for the more dominant role of the PT receptor cell field in the maintenance of the gaping response lies in the more prominent expression of T2R receptors \((\text{which bind bitter-tasting ligands})\) in the PT relative to the AT \((1)\). Presumably, upon reinnervation of the receptor cell field, some fibers in the reinnervating nerve \((\text{either the GL or CT})\) become functionally connected to these bitter ligand-activated receptor cells, which when stimulated by quinine, then provide sufficient neural activity to drive the gaping response. Because of the paucity of T2R receptors in the AT, the neural signal cannot reach a critical “threshold” no matter which nerve reinnervates that field.

This interpretation presupposes the existence of a subset of fibers in the CT that as a rule, normally provides input, like the GL, into the neural circuits controlling unconditioned oromotor rejection reflexes. CT nerve fibers innervating their native receptor cell field in the AT in conjunction with an intact palatal taste receptor field are capable of maintaining other types of quinine-stimulated behaviors \([\text{e.g., sensory discriminations in a normal fashion (44, 46, 47) and even gapes to sucrose conditioned to be aversive, when GL input is removed (10)}]\), but unconditioned gaping to quinine is by no means normal \((18, 27, 49)\). Nonetheless, previous findings lend credence to the suggestion that afferent axons in the CT are capable of mediating some aspect of quinine-stimulated gaping behavior. For example, as previously mentioned, bilateral GL
transection (CT intact) has been shown to dramatically attenuate the number of gapes to quinine (18, 27, 49), but they were not eliminated entirely. Moreover, bilateral CT transection (GL intact) decreased the numbers of quinine-stimulated Fos-positive cells throughout the gNST, albeit not as unequivocally as GL transection (28). These findings, along with the electrophysiological results of Oakley (36), suggest that when the “quinine-responsive” CT fibers innervate the PT taste cells

Fig. 5. Cross-regeneration of the gustatory nerves differentially affected the spatial distribution of quinine-stimulated Fos-labeled neurons in the gNST. The proportion of Fos-labeled neurons (means ± SE) in each subfield for different stimulus and nerve status conditions is shown. Q-SHAM (A) and SHAM-W (B) rats expressed distinctly different patterns of activity in the nuclei. The “typical” quinine pattern is represented by gray bars; the “typical” water pattern is represented by white bars. In control rats, the most discriminating feature between the patterns of activity produced by stimulation with quinine vs. water is the proportion of activated neurons in medial dorsal subfield (MD) (compare arrows). Quinine-stimulated animals lacking innervation of the PT receptor field. D: CT→AT; F: GL→AT; both elicit patterns of quinine-stimulated activity that is virtually identical to that observed in water-stimulated rats. On the contrary, animals with only innervation of the PT receptor field do show a typical quinine-stimulated pattern if the receptor field is reinnervated by the native GL nerve (GL→PT, C). If the PT is cross-wired with the CT nerve (CT→PT, E), however, the pattern of neuronal activity (cross-hatched bars) falls in between those seen for the quinine- and water-stimulated sham groups. LD, lateral-dorsal; MidD, middle-dorsal; MD, medial-dorsal; LV, lateral-ventral; MidV, middle-ventral; MV, medial-ventral (see Ref. 51).

Fig. 6. Cross-regeneration of the gustatory nerves differentially affected brain stem neuronal activation in the waist area, the classic taste region of the PBN. Photomicrographs of the left PBN show the characteristic Fos labeling in the subnuclei that comprise the waist area: the ventral lateral subdivision (black arrow), the central medial subdivision (white arrow), and the cell bridges in between in SHAM-Q (A), SHAM-W (B), CT→PT (C), and GL→AT rats (D). With regeneration of the CT nerve into the PT (CT→PT), the quinine-stimulated Fos-labeling in the waist area appeared similar to that observed in quinine-stimulated control rats (SHAM-Q). On the contrary, in rats with regeneration of the GL nerve into the AT (GL→AT), quinine-stimulated Fos-labeling appeared more like that observed in water-stimulated control rats. Scale bar = 250 μm.
It is important to note that the second-order neurons themselves do not necessarily have to be the site of the convergence; instead, interneurons may provide communication between these pathways. Second, some cells in the gNST (52) and in the PBN (20) respond to stimulation of both the AT and PT fields. These findings led the authors to suggest that the cross-modal projection mediated visual behavior. Though significantly more Fos-labeled neurons were found in these two external subdivisions in sham-operated animals following intraoral stimulation, response to quinine stimulation regardless of the nerve providing the input. Though significantly more Fos-labeled neurons were found in these two external subdivisions in sham-operated animals following intraoral stimulation with quinine compared with water, none of the nerve manipulations appeared to produce a significant effect on the quinine-stimulated Fos activity.

A caveat in our interpretation, however, stems from what is meant by adequate stimulation. For example, what if the cross-regenerated GL (into the AT) could somehow be adequately stimulated by the taste receptor cells located there? It is possible, in this case, that gaping would be elicited by quinine, and thus input from the AT receptor field would be “sufficient” to maintain the rejection behavior. The concentration of quinine used in this experiment was quite high from a behavioral standpoint (~3 orders of magnitude above the psychophysical detection threshold), but conceivably, even higher concentrations of quinine might have effectively generated gapes in rats that had the GL cross-wired into the AT. Nonetheless, the current results do distinguish the PT as playing a special role in mediating unconditioned gaping behavior. The preferential involvement of the PT in this function likely stems from its possession of the proper receptor cells successfully innervated by the proper type of nerve fibers, both of which are likely critical in restoring this behavior upon reinnervation of taste buds.

The interpretation that we have offered to explain the current outcomes, though simple and compelling, may undervalue the very complex and remarkable means by which functional reconnections between nerve fibers and taste receptor cells likely occur in the lingual epithelium. Even in normal rats, the mechanisms that guide appropriate functional connectivity in the tongue are unknown. It is widely assumed that because taste receptor cells undergo constant turnover while perceptual stability is maintained (5, 11), there must be a matching mechanism that allows gustatory fibers to couple with their native taste receptor cells in such a way that signals are appropriately channeled to brain circuitry to maintain function (35). Such a process is notable considering the fact that the axons comprising a taste nerve represent a heterogeneous population: some fibers respond to sweeteners, some to bitter compounds, some to sodium or other salts, some to acids (see Ref. 43 for a review). In short, not all axons are functionally the same within any taste nerve or across taste nerves. The current findings imply that a comparable and even more striking matching mechanism operates when a regenerating taste nerve (with its own distinct set of fibers) invades a foreign taste receptor cell field.

That the gustatory system was able to mediate a normal taste-relation behavior via experimentally induced novel reconnections between nerve fibers and taste receptor cells suggests that a comparable and even more striking matching mechanism operates when a regenerating taste nerve (with its own distinct set of fibers) invades a foreign taste receptor cell field. In this case, experimentally rewired CT nerve fibers were able to maintain gaping behavior when they reinnervated taste cells in a non-native receptor field in the PT. That the gustatory system was able to mediate a normal taste-related behavior via experimentally induced novel projections from the tongue (at least the PT) is testimony to the plasticity inherent within the system.

Though the “signal strength” explanation for the observed effects of cross-wiring on gaping behavior enjoys the benefit of parsimony, we cannot completely dismiss the possibility that our manipulations of peripheral gustatory input somehow triggered central reorganizational events (e.g., 31, 33) that played a role in the maintenance, or lack thereof, of quinine-elicited gaping. Reorganization of central structures as a result of cross-regeneration is not without precedence. Sur and colleagues (48) redirected retinal projections to the auditory thalamus in neonatal ferrets, and later, these animals possessed visually responsive cells in the auditory thalamus and cortex. Moreover, the cross-modal projection mediated visual behavior (56). These findings led the authors to suggest that the function of central visual structures might be specified by their
extrinsic inputs. Our findings hint at the possibility that such a
scenario might play out in the gustatory system as well. It is
within reason to speculate that gNST cells in the cross-wired
animals experience neural signals to which they are not nor-
amally accustomed. In response to the new extrinsic inputs, they
may rearrange their connections to sustain normal oromotor
rejection behaviors. It is critical to stress, however, the impor-
tant differences between our work and the work of Sur and
colleagues (48): 1) their experimental cross-wirings were per-
formed on neonates, whose brains are typically more plastic
than those of the adult rats used in our study; and 2) the authors
were looking at forebrain structures that also are generally
accepted as being more malleable than hindbrain structures.
Cautions notwithstanding, there is evidence of central anatom-
cal consequences of gustatory nerve transection (59), and thus,
although we favor the more straightforward explanation, it
would be imprudent for us to completely reject central reorga-
nization as a candidate mechanism.

Anatomical topography for oromotor reflexes. Previous an-
amotomical studies have shown that quinine elicits a distinct
distribution of Fos activation within the gNST (24) that is
maintained across concentrations of this tantant (51) and by
different bitter stimuli (7). Because this pattern of Fos expres-
sion is somewhat distinct from those observed following suc-
rose or water stimulation, for example, Harrer and Travers
(24) suggested the existence of a rough stimulus-based spatial
organization (or chemotopy) within the gNST. However, be-
cause of accumulating evidence linking these particular bitter
ligand-responsive cells to reflex rejection, Travers and Travers
(53) more recently proposed that the Fos-activated cells in MD
following quinine stimulation represent a novel type of orga-
nization within the gNST—a reflex topography. The authors
acknowledge that stimuli that produce rejection reflexes are
often bitter, but because reflex rejection is just one function of
bitter tastants, they make the case that “reflex topography” is
more precise here than “chemotopy.”

Although the neural circuit underlying reflex rejection is not
completely understood, the gNST and the waist area (but not
the external subdivisions) of the PBN have been implicated as
part of the neural substrate involved (50). Our previous find-
ings, as well as the current ones, lend support for this hypo-
thesis. We have shown before that GL transection greatly attenu-
ates the numbers of quinine-stimulated Fos neurons in these
two taste-responsive regions (MD and the waist area) and that
regeneration of the GL restores these numbers in parallel with
recovery of gaping behavior (26, 27, 28). In these studies, the
Fos response (either attenuation or restoration) in MD was
observed at each rostrocaudal level of the gNST analyzed and
therefore suggested that MD neurons throughout the gNST
were associated with the neural circuits underlying rejection
reflexes. The current findings, however, call attention specifi-
cally to MD neurons in the rostral-most gNST, because in all
groups that demonstrated significant and statistically similar
numbers of gapes to quinine (e.g., those with PT reinnerva-
tion), there were nearly equal numbers of Fos-labeled neurons
in the rostral-most MD subfield, and these were significantly
greater than those observed in groups that exhibited few gapes.
In the more caudal levels of the gNST, such relationships were
not observed. Accordingly, it is reasonable to speculate that
this particular subpopulation of neurons in MD, along with the
waist area of the PBN, may serve a specialized role in the
neural circuit underlying gustatory rejection behaviors; but it
remains to be seen as to what extent these relationships are
causal.

Perspectives and Significance

The importance of the current findings is highlighted by
close to 20 years of nerve transection and behavioral work
across a variety of laboratories, including ours, that suggests
that gustatory nerves in rodents are to some extent functionally
specialized. Indeed, it is accepted in the taste literature that the
GL plays a privileged role in unconditioned gaping to aversive
compounds. The present findings place limitations on this view
and demonstrate that the signals in the CT are capable of
triggering these responses when the fibers of this nerve connect
themselves to the PT taste buds.

To our knowledge, these results are the first in which the
consequences of the cross-wiring of cranial sensory nerves
have been simultaneously assessed on behavior and neural
activity in an adult mammalian model. It is remarkable that
under our cross-wired conditions normal taste-elicited rejection
reflexes were restored. There was no a priori reason to expect
a favorable behavioral outcome in any rewired state in these
adult animals; the most parsimonious prediction would have
been that none of the cross-reinnervation conditions would
support normal gaping to quinine. This, however, was not the
case. Rather, the gustatory system was able to adapt successfully
to peripherally reorganized input, at least with respect to the
specific reflex rejection behavior measured here. In the future,
it will be important to test whether other taste-related functions
interrupted by lingual gustatory nerve transection can be maintai-
 ned under conditions in which the nerves are cross-wired. The com-
 pilation of such results should help to open new and important
questions about the scope of plasticity within the gustatory sys-
tem, as well its potential to recover from nerve injury.

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