Nerve regeneration-induced recovery of quinine avoidance after complete gustatory deafferentation of the tongue

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Geran, Lauren C., Mircea Garcea, and Alan C. Spector. Nerve regeneration-induced recovery of quinine avoidance after complete gustatory deafferentation of the tongue. Am J Physiol Regul Integr Comp Physiol 287: R1235–R1243, 2004; doi:10.1152/ajpregu.00137.2004.—The concentration-dependent decrease in quinine licking by rats is substantially attenuated by combined bilateral transection of the chorda tympani (CT) and glossopharyngeal (GL) nerves, but transection of either nerve alone produces marginal impairments at most. Here we tested whether regeneration of one or both of these nerves after combined transection would result in recovery of taste avoidance. Water-restricted rats were presented with a series of brief-access (5 s) taste trials (water and 0.003–3.0 mM quinine-HCl) in a 5-day test block of 40-min sessions both before nerve transection and starting 75–77 days after transection. Licking avoidance returned to presurgical levels when both nerves were allowed to regenerate. When only the GL was allowed to regenerate, performance did not differ from that of sham-transected animals. This suggests that even after considerable gustatory deafferentation, regeneration has the capacity to restore normal taste-guided behavior. Surprisingly, when only the CT was allowed to regenerate, avoidance behavior was severely impaired and was not different from that of rats in which regeneration of both nerves was prevented. Taking into account prior findings, it appears that the absence of the GL in the presence of an intact CT is fundamentally different from the absence of the GL in the presence of a regenerated CT with respect to some taste functions. This represents the first reported instance to our knowledge in which the capacity of a regenerated nerve to maintain taste-guided behavior was distinctly different from that of an intact nerve in a rodent model.

taste; chorda tympani nerve; glossopharyngeal nerve; avoidance behavior; neural plasticity

CHORDA TYMPANI (CT) and glossopharyngeal (GL) axons are able to regenerate when severed and eventually reinnervate their appropriate lingual receptor fields. The amount of reinnervation that takes place depends on several factors, including the species, how and where the injury is made, and whether surgical anastomosis is performed (see 5, 8, 19, 20, 23, 24, 34, 42, 43). In rodents, the CT and GL can be completely transected and, without any further intervention, find their way back to their native receptor fields, unless a large section of the nerve is removed or neurotoxins are specifically applied (2, 7, 13, 15, 16, 21, 34, 36, 40). Although significant regeneration occurs, only about three-fourths of the normal complement of taste buds appear to return, and the volume of the regenerated taste buds is reduced by >40% (13, 16, 25, 34, 36). This raises the question of whether taste function returns to presurgical levels after a periodic loss of peripheral input and a long-lasting if not permanent reduction in the number and volume of taste buds on lingual reinnervation.

To date, only a handful of studies have explored the effects of gustatory nerve regeneration on taste-guided behavior. Hamsters that have successfully acquired a conditioned taste aversion to NaCl subsequently fail to avoid the salt solution after CT transection but can reacquire the aversion on regeneration of the nerve (3). In rats, CT transection produces an increase in NaCl detection threshold of >1 order of magnitude and impairs the ability to discriminate NaCl from KCl, with sensitivity and salt discriminability returning to presurgical levels once the CT has regenerated (15, 16). Moreover, in contrast to CT-transected rats, animals with regenerated CTs are just as sensitive to the performance-disrupting effects of amiloride treatment on NaCl detectability as intact rats (16). These findings suggest that regeneration of the CT leads to normal recovery of salt taste in rodents. The effects of CT regeneration on the perception of “sweet” or “bitter” compounds have not been examined primarily because transection of this nerve has not produced remarkable deficits in behavioral tasks involving these taste stimuli unless combined with neurotoxins of other gustatory nerves (26, 29–33, 37, 41).

Similarly, it is not meaningful to examine functional recovery after regeneration of the GL because transection of this nerve alone does not lead to any substantial taste deficits as assessed behaviorally, with one major exception. This manipulation markedly reduces the number of unconditioned gapes and other reflex-like rejection responses elicited by quinine (10, 13, 39). Interestingly, CT transection has little effect on aversive taste reactivity to quinine. When the GL regenerates, gaping returns to normal despite a decrease in the number of circumvallate taste buds (13). In addition, the characteristic pattern of Fos-like immunoreactivity (FLI) stimulated by quinine in the rostral nucleus of the solitary tract (NST) (see Refs. 11, 38) and in the parabrachial nucleus (PBN) that is disrupted by GL transection returns to normal on regeneration of the nerve (12, 13).

Although in rats transection of the GL alone results in a marked reduction in the number of gapes elicited by intraorally delivered quinine solutions, it has little effect on unconditioned lick avoidance as assessed in brief-access taste tests of quinine responsiveness (33, 41). In the brief-access taste test, rats are presented with a repeating series of very short (a few seconds) periods of access to taste stimuli, and the degree of licking is quantified. The difference in the effect of GL transection on quinine-elicited gaping vs. brief-access lick avoidance suggests that gustatory circuits involved in reflex-like oromotor rejec-
tion responses can be functionally dissociated from those involved with approach behavior and maintenance of spout licking, at least with respect to certain stimuli.

Quinine concentration-response functions derived from brief-access licking tests are substantially shifted to the right, however, after transection of both the GL and CT (33). This combined neurotomy leads to degeneration of most of the taste buds of the tongue and reduces the total number of taste buds in the oropharyngeal cavity by close to 75% (see Refs. 18, 27). Thus the GL does contribute to lick avoidance responses to quinine, but in a more complex way than originally anticipated. Either a threshold number of taste receptors must be removed before taste deficits involving quinine are revealed in this task, or the input from the two nerves converges centrally such that signals from one nerve are sufficient to maintain normal performance. Either of these hypotheses, which are not mutually exclusive, could explain why combined transection of the CT and GL results in severe blunting of quinine lick avoidance, whereas transection of either nerve alone is without effect. It is noteworthy that combined transection of the CT and GL produces a minor effect at best on brief-access responses to maltose, sucrose, and NaCl (see Ref. 27). Thus the attenuation of lick avoidance to quinine after this particular manipulation of peripheral taste input displays some degree of chemospecificity, with the caveat that only a few compounds have been tested.

The purpose of this study was to test whether normal concentration-dependent lick avoidance of quinine in a brief-access test would return on regeneration of the CT and GL after the combined transection of these nerves. Given that gaps and lick avoidance in response to quinine can be dissociated based on the effects of nerve transection, it is unclear whether the observation that oromotor rejection reflexes recover on GL regeneration would extend to restoration of normal licking responses in the brief-access test after complete gustatory deafferentation of the tongue. The brief-access test provides a convenient and effective way to assess taste responsiveness in the suprathreshold concentration range. This methodology allowed us to address the question of whether regeneration-induced recovery of taste function was possible after a more massive insult to the peripheral gustatory system; the prior studies of regeneration-induced recovery of taste-guided behavior have involved the transection of only a single gustatory nerve. We also assessed performance when only the CT or the GL was allowed to reinnervate its target after their combined transection. Previous brief-access experiments have shown that transection of either nerve alone results, at most, in only minor alterations in quinine concentration-response function, thus enabling us to compare the sufficiency of the regenerated nerve with that of the intact nerve in maintaining avoidance.

METHODS

Subjects

Forty naive adult male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) were used in two phases of 20 rats each. These animals weighed 246–288 g at the start of training and were housed individually in hanging wire-mesh cages. Temperature, humidity, and light cycle (12:12-h light/dark) were controlled automatically with lights on at 7:00 AM. Rats were given ad libitum access to chow (PMI 5001 pelleted chow, PMI Nutrition International, Brentwood, MO) in their home cages. Access to water, however, was restricted to weekends and sessions in the apparatus. Distilled water bottles were removed from the home cages each Sunday no more than 24 h before testing. Bottles were replaced after the last session each Friday afternoon. During the week, rats received fluid access only while in the apparatus. Body weight was closely monitored to ensure that no rat dropped below the 85% body weight calculated each week. If an animal’s weight dropped below this level, it received 5 ml of supplemental water. All methods involving laboratory animals were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Apparatus

Rats received training and testing in a gustometer. This computer-controlled apparatus was designed to record licking in response to small volumes of taste stimuli delivered during brief trials (see Ref. 28). The test chamber had an access slot on one wall through which the rat could reach a stainless steel ring on the tip of a Kel-F drinking spout with its tongue. This spout was connected by way of Teflon tubing to pressurized reservoirs filled with taste stimuli. Each trial was initiated when the rat licked the dry spout twice within 500 ms. This criterion was included to ensure that the rat was actively licking the spout before stimulus delivery. Once this criterion was met, the shaft of the spout filled with the stimulus, and each subsequent lick resulted in the delivery of 5 μl of fluid. Each trial lasted 5 s. Each time the rat contacted the spout, an electrical circuit was closed (<50 nA) and registered as a lick by a computer. After each trial, the spout rotated over a funnel out of the subject’s reach and was rinsed with distilled water and blown dry with pressurized air. The gustometer was housed within a sound attenuation cubicle (BRS-LVE, Laurel, MD), and white noise was presented throughout the session to minimize potential auditory cues related to stimulus delivery.

Gustometer Training and Presurgical Testing

Rats were given 5 days of spout training with distilled water as the only stimulus to promote habituation to the apparatus and stimulus delivery protocol. During the first 2 days of training, rats were given 30-min access to distilled water in the chamber with fluid delivered whenever the rat made contact with the spout. On the following 3 days of training, sessions were 40 min long, and fluid was delivered in 5-s bouts only when the 500-ms interlick interval criterion was met, thus mimicking testing conditions. After training, rats were given 5 days of presurgical testing with the seven concentrations of quinine hydrochloride (3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003 mM) and distilled water in 40-min test sessions. In addition to these eight stimuli, the animals were also given a distilled water rinse trial between test stimulus trials to minimize adaptation and carryover effects and to encourage trial initiation. Stimuli were prepared fresh each morning using reagent-grade chemicals (Sigma-Aldrich, St. Louis, MO).

Surgery and Recovery

Rats were divided into five groups with eight rats each such that there were no significant differences (i.e., P > 0.05) in the number of trials initiated, body weight, or the quinine concentration at which the ratio of licks to the tastant was one-half the number of licks to water (c value, see Data Analysis). All surgery was performed after inducing anesthesia with an intramuscular injection of ketamine (Ketaset, 125 mg/kg body wt) mixed with xylazine (Rompun, 5 mg/kg). Bilateral nerve transection was performed using two different methods, one in which regeneration was prevented (X) and one in which it was allowed (R). The five groups were CT(R) + GL(R), CT(R) + GLX, CTX + GL(R), CTX + GLX, and sham-operated control. Regeneration of the GL was prevented by removing ~10 mm of this nerve (13), while regeneration of the CT was prevented by cauterizing the nerve, a portion of the malleus, and the junction of the ear canal and tympanic membrane. Regeneration was allowed by simply cutting each nerve with microscissors once it was exposed (see Refs. 16, 33).

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The CT was exposed by retracting the pinna and removing the tympanic membrane with forceps. The GL was exposed by making a midline incision in the ventral neck and retracting the salivary glands, the omohyoid and sternohyoid muscles, the hyoid, and the posterior belly of the digastric muscle. In the sham surgery group, the tympanic membrane was punctured and the GL was exposed but otherwise untouched. Wounds were closed with sterile nylon sutures. Each rat was given subcutaneous injections of penicillin (Floccillin, 30,000 U) and an analgesic (Ketorolac, 2 mg/kg body wt) immediately after surgery and for the next 3 days. During this period, the animals were also given wet mash made with powdered PMI 5001 chow and Nutri-Cal (Evşco Pharmaceuticals, Buena, NJ) regardless of surgery group. Rats were maintained on the mash on an individual basis until they reached 80% of their presurgical body weight. This took no more than 6 days. Animals recovered in their home cages for 68–70 days before the start of postsurgical testing. Three animals died immediately after surgery. One of these rats was from the CT(R) + GLX group (resulting sample size: n = 7) while the other two were from the CTX + GLX group (resulting sample size: n = 6). Presurgical data from these animals were not included in the analyses.

**Post-surgical Testing**

Subjects were tested in the same manner as they were presurgically. This included 5 days of spout training with distilled water followed by 5 days of quinine testing. After testing, the rats were deeply anesthetized with an overdose of pentobarbital sodium and rapidly perfused with saline and buffered formalin (10%). Tongues were collected and stored in formalin until histology could be performed.

**Histology**

All histological analysis was performed blind to surgery group. The anterior portion of each tongue was separated from the posterior tongue and placed in distilled water for 1 h and then dipped in 0.5% methylene blue for ~1 min. The tissue was then rinsed and dried, and the epithelium was separated from the underlying connective and muscle tissue and flattened between two glass slides. The number of visible fungiform papillae and taste pores was counted under a light microscope. A pore was counted as present if a dark blue circle was visible on the dorsal surface of a fungiform papilla (22, 35). This analysis has been used previously and correlates well with regeneration of the CT and the associated recovery of function on salt detection and discrimination tasks (15, 16, 36).

The tissue of the circumvallate and foliate regions of the posterior tongue was removed and embedded in paraffin (24, 192). This incision has been used previously and correlates well with regeneration of the CT and the associated recovery of function on salt detection and discrimination tasks (15, 16, 36).

The tissue was then sliced on a microtome into 10-μm sections, placed on slides, and stained with hematoxylin and eosin. Morphologically intact taste buds were identified by either the presence of a taste pore or of well-organized conical cells converging near the epithelium. Taste pores appeared as clear ovals at the apex of the taste bud or as conduits through the epithelium. A pore was counted as present if a dark blue circle was visible on the dorsal surface of a fungiform papilla (22, 35). This analysis has been used previously and correlates well with regeneration of the CT and the associated recovery of function on salt detection and discrimination tasks (15, 16, 36).

**Data Analysis**

A lick ratio score was derived for each animal at each concentration by dividing the average number of licks for each quinine concentration by the average number of licks to distilled water collapsed across the 5 days of testing. A lick ratio score of 1.0 would indicate that the rat licked equivalently to the taste stimulus and to water while a score of 0.0 would mean that the rat failed to lick the tastant. A score of 0.5 would mean that the rat licked one-half as much for the quinine stimulus as for water. Two-parameter logistic functions were then fit to the data using the following equation:

\[ f(x) = \frac{1}{1 + 10^{(x - c)}} \]  

where \( b \) = slope, \( x = \log_{10} \) quinine concentration, and \( c = \log_{10} \) quinine concentration at which the lick ratio score was equal to 0.5.

The data were analyzed with one-way and two-way ANOVAs, as well as post hoc analyses when appropriate. Both paired and independent \( t \)-tests were also performed in addition to Pearson’s correlations comparing the results of the histology with quinine avoidance performance. The conventional \( P \leq 0.05 \) was used as the criterion for statistical significance.

**RESULTS**

**Lick Ratio Scores**

A two-way ANOVA (group × concentration) of the lick ratio scores failed to indicate a significant difference in presurgical quinine avoidance between groups [\( F(4,32) = 0.11, P > 0.97; \) Fig. 1]. After surgery, however, the group effect was significant [\( F(4,32) = 7.6, P < 0.001 \)]. Both ANOVAs were significant for concentration [\( F(6,192) > 264, P < 0.001 \)], and the group × concentration interaction was significant after surgery [\( F(24,192) = 12.7, P < 0.001 \)]. A series of two-way ANOVAs (concentration × pre- vs. postsurgery) was performed to determine which groups had a significant change in performance. These statistics revealed a significant effect of concentration for each group (\( F > 80, P < 0.001 \) for each) and significant surgery effects for the CT(R) + GL(R), CT(R) + GLX, and CTX + GLX groups (\( F > 6.4, P < 0.05 \) for each). Surgery × concentration interactions were significant (\( F > 5.6, all P < 0.001 \) for all but the CT(R) + GL(R) group. Although there was a significant main effect of surgery and/or a surgery × concentration interaction in the sham-operated, CT(R) + GL(R), and CTX + GLX groups, the magnitude of these shifts was relatively minor compared with those in the other two groups [CTX + GLX and CT(R) + GLX; Fig. 2].

**Concentration at lick ratio score = 0.5 (c value).** The mean concentration representing \( c \) from the individual curve fits for

\[ \text{LICK RATIO SCORE} \]

\[ \text{QUININE CONCENTRATION (mM)} \]

\[ \text{CTX(R) + GLX} \]

\[ \text{CTX} + \text{GLX} \]

\[ \text{CTX} + \text{GL(R)} \]

\[ \text{SHAM} \]

Fig. 1. Mean (±SE) lick ratio score as a function of quinine concentration assessed presurgically for each group. Curves were fit by a 2-parameter logistic function (see text). There were no significant differences between groups. Sham, sham-operated control group. All of the remaining groups had both the chorda tympani nerve (CT) and glossopharyngeal nerve (GL) transected, and regeneration was either allowed (R) or prevented (X).
all rats before surgery was approximately $-0.27 \log_{10}$ units ± 0.05 (0.53 mM). One rat (rat 3) in the CTX + GLX group was removed from analyses of curve parameters because the concentration-response function was flat and a logistic curve could not be fit to the data. An ANOVA of the change in the $c$ value with surgery indicated a significant effect of group $[F(4,31) = 11.5, P < 0.001]$. A Bonferroni post hoc analysis showed that while the CTX + GLX and CT(R) + GLX groups were not different from one another, they were different from each of the other groups ($P < 0.003$ for each), which in turn were not different from each other. Paired $t$-tests indicated no change in $c$ concentration with surgery (Figs. 2 and 3) for rats in the sham-operated, CT(R) + GL(R), and CTX + GL(R) groups ($P$ values > 0.09). The concentration-response function shifted markedly, however, for rats in the CTX + GLX ($1.35 \log_{10}$ units ± 0.32, $P < 0.002$) and CT(R) + GLX groups ($1.3 \log_{10}$ units ± 0.86, $P < 0.008$). It should be noted, however, that the flattened shape of the curves from the CTX + GLX and CT(R) + GLX groups made it necessary to extrapolate $c$ values to a large degree, making them less accurate than the values from the other groups. Nonetheless, even without exact parameter values, it is clear from Fig. 2 that rats in these groups markedly increased licking at the highest concentration after surgery.

**Slope.** One-way ANOVAs failed to indicate a group effect for slope ($b$ value) either before $[F(4,31) = 1.59, P > 0.2]$ or after $[F(4,31) = 1.6, P > 0.19]$ surgery. Likewise, the change in slope with surgery failed to reach significance $[F(4,31) = 1.75, P > 0.16]$. Although the functions for both the CTX + GLX and CT(R) + GLX groups flattened considerably after surgery (Fig. 2), the $b$ values (Table 1) did not differ significantly from presurgical values ($P > 0.07$). This lack of significance may have been due in part to the low sample sizes for each of these groups [CTX + GLX: $n = 5$; CT(R) + GLX: $n = 7$] or may have been a consequence of the extrapolation due to the substantial decrease in sensitivity (i.e., shift in the $c$ value) (Fig. 2). Rats in the sham-operated and CT(R) + GL(R) groups also failed to show a significant change in slope with surgery ($P > 0.69$). In contrast, the mean $b$ value for rats in the CTX + GL(R) group decreased significantly after surgery, although the magnitude of this change was relatively minor (difference = 0.25, $P < 0.02$) as is evident in Fig. 2.

![Fig. 2. Mean (±SE) lick ratio score as a function of quinine concentration assessed before (●) and after (○) surgery for each group. The curves were fit by a 2-parameter logistic function (see text). Groups where GL regeneration was prevented [CTX + GLX and CT(R) + GLX] demonstrated a significant shift in the concentration-response function ($P < 0.008$ for each).](image-url)
Total Licks

Before surgery, the groups did not differ with regard to the number of licks during water trials \( F(4,32) = 0.19, P > 0.94 \) but did so after surgery \( F(4,32) = 4.3, P < 0.008 \). A post hoc analysis showed that the CTX + GL(R) licked water significantly less than did either the CT(R) + GLX \( P < 0.05 \) or sham-operated \( P < 0.007 \) groups. Furthermore, a series of paired \( t \)-tests indicated that surgery significantly changed the number of licks to water for only the CTX + GL(R) group \( P < 0.007 \); Bonferroni adjusted \( P < 0.035 \); Table 2).

These data suggest that the attenuating effects of surgery on quinine avoidance in the CTX and CTX + GLX groups were not due to a general decrease in licking competence.

Additionally, a one-way ANOVA of body weights taken on the day before postsurgical testing began showed no effect of group \( F(4,32) = 0.58, P > 0.67 \), suggesting that rats in the gustatory nerve transection/regeneration groups were able to eat and drink essentially as well as those in the sham-operated control group.

Histology

Oral tissue from one rat in the CT(R) + GL(R) group could not be salvaged, reducing the sample size to seven for each papillary field. A one-way ANOVA of taste pores in the fungiform papillary field indicated significant differences across groups \( F(4,31) = 166.7, P < 0.001 \). A Bonferroni post hoc analysis revealed that the sham-operated group had significantly more taste pores than any of the other groups \( P < 0.002 \), Fig. 4). The CT(R) + GLX and CTX + GLX groups were not different from one another \( P > 0.38 \) but did have significantly more pores than either the CTX + GLX or CTX + GL(R) group \( P < 0.001 \) for each. The two groups in which CT regeneration was prevented were also not different from one another in terms of number of visible taste pores in the anterior tongue \( P > 0.99 \). These findings confirm that the

![Fig. 3. Surgically mediated shifts (postsurgery – presurgery) in the log10 concentration at which the lick ratio score equaled 0.5 (c value) based on the curve fits for each rat followed by group mean (M). All shifts are in log10 units. Bars directed upward represent rightward shifts in the concentration-response function after surgery. # Curve could not be fit to the data for this animal. Only the CTX + GLX and the CT(R) + GLX groups displayed statistically significant shifts \( p < 0.05 \).](image)

Table 1. Curve fit parameters (lick ratio scores)

<table>
<thead>
<tr>
<th>Surgery Group</th>
<th>Presurgical Slope (b)</th>
<th>Post Surgical Slope (b)</th>
<th>P Value (b)</th>
<th>Presurgical c-Concentration, M</th>
<th>Post Surgical c-Concentration, M</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.70 ± 0.07</td>
<td>0.74 ± 0.13</td>
<td>( P &gt; 0.68 )</td>
<td>(-0.33 ± 0.2 \log_{10} )</td>
<td>(-0.23 ± 0.3 \log_{10} )</td>
<td>( P &gt; 0.52 )</td>
</tr>
<tr>
<td>CT(R) + GL(R)</td>
<td>0.89 ± 0.07</td>
<td>0.91 ± 0.08</td>
<td>( P &gt; 0.77 )</td>
<td>(-0.25 ± 0.1 \log_{10} )</td>
<td>(-0.07 ± 0.1 \log_{10} )</td>
<td>( P &gt; 0.09 )</td>
</tr>
<tr>
<td>CTX + GL(X)</td>
<td>0.81 ± 0.06</td>
<td>0.56 ± 0.08</td>
<td>( P &lt; 0.02 )</td>
<td>(-0.24 ± 0.1 \log_{10} )</td>
<td>(-0.03 ± 0.1 \log_{10} )</td>
<td>( P &gt; 0.10 )</td>
</tr>
<tr>
<td>CT(R) + GLX</td>
<td>0.86 ± 0.05</td>
<td>0.69 ± 0.12</td>
<td>( P &gt; 0.27 )</td>
<td>(-0.27 ± 0.1 \log_{10} )</td>
<td>(1.03 ± 0.3 \log_{10} )</td>
<td>( P &lt; 0.008 )*</td>
</tr>
<tr>
<td>CTX + GLX</td>
<td>0.81 ± 0.04</td>
<td>0.73 ± 0.05</td>
<td>( P &gt; 0.07 )</td>
<td>(-0.17 ± 0.2 \log_{10} )</td>
<td>(1.18 ± 0.1 \log_{10} )</td>
<td>( P &lt; 0.002 )*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sham, sham-operated control group. All of the remaining groups had both the chorda tympani nerve (CT) and glossopharyngeal nerve (GL) transected, and regeneration was either allowed (R) or prevented (X). c, \( \log_{10} \) quinine concentration at which lick ratio score was equal to 0.5. * Significant difference, pre- vs. postsurgery value.
CT did regenerate in the CT(R) groups and did not in the CTX groups.

Analysis of the circumvallate papilla showed a similar pattern $[F(4,31) = 103.7, P < 0.001]$ with sham-operated rats and rats in the CTX + GL(R) group having the greatest number of taste buds. Although the sham-operated animals had significantly more taste buds than the CT(R) + GL(R) group ($P < 0.04$), they did not significantly differ from the CTX + GL(R) group ($P > 0.12$). Groups for which the GL was allowed to regenerate had significantly more taste buds than the groups in which the GL was prevented from regenerating ($P < 0.001$ for each). Groups without GL regeneration were not different from one another ($P > 0.99$). Analysis of the foliate papillae also indicated group differences $[F(4,28) = 30.9, P < 0.001]$; no differences were found among the sham-operated, CT(R) + GL(R), and CTX + GL(R) groups using a Bonferroni analysis ($P > 0.99$ for each). The CT(R) + GLX and CTX + GLX groups had very few taste pores and were not significantly different from one another ($P > 0.99$) but were different from the other three groups ($P < 0.001$). Unfortunately, foliate data from three rats in the sham-operated group were deleted from the analysis due to loss of tissue during preparation, resulting in a sample size of 5. Paired $t$-tests of the remaining animals indicated that there was no significant difference between the number of taste buds on the left after recovery and those on the right ($P > 0.17$ for each group) regardless of whether a Bonferroni correction was applied.

**DISCUSSION**

This study clearly replicated prior work showing that combined transection of the CT and GL severely disrupts concen-

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**Table 2. Total water licks**

<table>
<thead>
<tr>
<th>Surgery Group</th>
<th>Mean Presurgical Licks to Water</th>
<th>Mean Posturgical Licks to Water</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>32.3 ± 1.14</td>
<td>30.8 ± 1.03</td>
<td>$P &gt; 0.08$</td>
</tr>
<tr>
<td>CT(R) + GL(R)</td>
<td>30.1 ± 2.12</td>
<td>29.4 ± 0.89</td>
<td>$P &gt; 0.74$</td>
</tr>
<tr>
<td>CTX + GL(R)</td>
<td>31.6 ± 0.96</td>
<td>24.4 ± 1.96</td>
<td>$P &lt; 0.007^*$</td>
</tr>
<tr>
<td>CT(R) + GLX</td>
<td>31.2 ± 1.48</td>
<td>29.7 ± 0.58</td>
<td>$P &lt; 0.31$</td>
</tr>
<tr>
<td>CTX + GLX</td>
<td>31.6 ± 3.50</td>
<td>29.2 ± 0.96</td>
<td>$P &gt; 0.41$</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Significant difference, pre- vs. postsurgery value.

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**Fig. 4.** Mean ($±$SE) number of taste pores (anterior tongue) or taste buds counted for each group and papillary field. Horizontal bars indicate that the counts for the groups located underneath were not significantly different (i.e., $P > 0.05$).

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**Correlations.** The CT(R) + GLX group exhibited the greatest variability in quinine avoidance with surgery (see Fig. 3). Data from this group were analyzed to determine whether this variability could be accounted for by differences in the number of taste buds/pores ($r = 0.77, P < 0.05$) for this group but not with any specific papillary field ($P > 0.15$ for each).
tion-dependent quinine avoidance as assessed in a brief-access taste test (33). More importantly, our results demonstrate that affective responsiveness to quinine, as assessed by this procedure, can be entirely restored after extensive gustatory deafferentation, provided sufficient nerve regeneration takes place. Interestingly, the CTX + GL(R) rats completely recovered their quinine avoidance behavior, but the CT(R) + GLX rats displayed significantly impaired performance. This latter finding was unexpected because transection of the GL alone has only modest effects at best on quinine avoidance in the brief-access test (17, 33, 41). The attenuation of quinine avoidance in the CT(R) + GLX group, however, was quite severe. Indeed there was no significant difference between the postsurgical performances of the CTX + GLX and CT(R) + GLX groups. Thus it appears that the absence of the GL in the presence of an intact CT is fundamentally different from the absence of the GL in the presence of a regenerated CT with respect to some taste functions. This represents the first reported instance to our knowledge in which the capacity of a regenerated nerve to maintain taste-guided behavior was distinctly different from that of an intact nerve in a rodent model.

There are several possible explanations for the functional disparity between the intact and the regenerated CT with respect to this paradigm. One general hypothesis is that in the absence of the GL, regeneration of the CT leads to changes in peripheral taste receptor cells that would otherwise not occur in the absence of an intact GL. For example, the CT(R) + GLX group had only 67% of the number of fungiform taste buds found in the sham-operated animals. As noted above, this reduction in taste bud number in the field of the regenerated CT has not precluded full recovery of taste-guided behaviors in other tasks. However, when the total taste bud population is already significantly abridged by the absence of another major gustatory nerve (i.e., the GL), a 33% reduction in the number of anterior tongue taste buds may have more severe functional consequences. In addition, it is possible that there are fewer taste receptor cells per taste bud (see Ref. 25) or a lower density of T2R receptors per taste receptor cell. The T2R family of G protein-coupled receptors has been shown to be critical in the transduction of “bitter” stimuli in the rat (see Refs. 4, 6). Indeed, any component of the normal transduction cascade could potentially be altered with regeneration. In hamsters, regenerated CT nerves were shown to contain ~67% fewer myelinated fibers than intact nerves (5). If this phenomenon also occurs in the rat, then such an effect might contribute to the inability of the regenerated CT to maintain normal quinine avoidance when the GL is transected. In fact, in rats with regenerated CTS, there is a 20% loss of geniculate ganglion cells (25).

Other potential mechanisms for the disparity in the behavioral effects observed between rats with an intact CT and those with a regenerated CT in the absence of posterior tongue taste receptors (i.e., GLX) may be central in origin. Perhaps the extensive loss of gustatory input resulting from the combined transection of the CT and GL triggers changes in the central gustatory system that compromise the ability of the input provided by the regenerated CT to maintain quinine avoidance behavior.

The central consequences of gustatory nerve injury and regeneration have only begun to be assessed. Although the topography of the CT terminal field in the NST was normal in hamsters with regenerated CTS, the area of the field decreased by ~23% (1). Recently, Cheon and Hill (9) reported a 67% reduction in the CT terminal field volume in rats after CT regeneration. In hamsters, CT transection does not result in a significant loss of geniculate ganglion cells, but there is evidence of fiber degeneration persisting in the NST even after CT regeneration (40).

Transsection of the GL changes the topographic pattern and number of NST neurons expressing FLI in response to quinine such that the pattern more closely resembles that of water stimulation (14). However, the distribution and number of neurons expressing FLI recovers to normal on regeneration of the GL (13). Transection of the CT reduces the number of NST neurons expressing FLI in response to intraoral quinine stimulation but does not alter their topographic pattern (14). At the level of the PBN, GL transection reduces the number of neurons in the “waist” area expressing FLI in response to intraoral quinine to a value similar to that seen in response to water, but CT transection is without effect. Neither nerve transection alters the number of quinine-induced FLI in the external subdivisions of the PBN (12). In rats with regenerated GLs, the number of FLI-neurons in the waist area stimulated by intraoral quinine infusion is similar to that of intact controls. Because transection of the GL alone (i.e., CT left intact) has only minor effects on quinine lick avoidance in brief-access tests, but causes striking decreases in gustes, which return to normal when the GL regenerates, it has been hypothesized that the FLI neurons observed in the brain stem gustatory nuclei contribute to neural circuits involved with reflexlike oromotor rejection responses (see Ref. 27).

Indeed, nerve transection and subsequent regeneration could lead to alterations in neuronal responsiveness and connectivity within any nucleus along the central gustatory pathway. Although there are some documented changes in the peripheral and central gustatory system associated with nerve regeneration, as noted above, it is difficult at this time to causally relate such findings to the specific behavioral outcomes in this study.

It is also possible that performance might have been compromised due to impaired salivary function with nerve transection that contains parasympathetic efferents that supply partial innervation to the sublingual and submaxillary salivary glands, and fibers from the GL innervate the von Ebner’s glands in the posterior tongue. This hypothesis is unlikely, however, due to the results of a previous study in which rats that underwent bilateral removal of both the sublingual and submaxillary salivary glands failed to show impaired quinine performance (33). Likewise, GL transection does not appreciably affect quinine avoidance in a brief-access test (33, 41, also see Ref. 17), suggesting that the innervation of the von Ebner’s glands is not essential for licking to be normally suppressed by this stimulus. We cannot entirely dismiss, however, that the consequences of these nerve transections on salivary function may be more severe over the extended recovery period used in the present study. Nevertheless, at present there is no strong evidence that salivary function is at the root of the behavioral effects described here.

Another possibility to consider is that the impairment in quinine avoidance observed in our CT(R) + GLX group was due to the length of time between pre- and postsurgical quinine testing combined with loss of GL input. In support of this interpretation, Markison and colleagues (17) reported that GL
transection in naive rats had a modest but significant effect on quinine avoidance in a brief-access test compared with the performance of sham-transected naive rats. Perhaps rats in the current experiment were functionally naive when tested after surgery because of the 75- to 77-day postsurgical recovery period. It should be noted, however, that the shift observed by Markison et al. (17) was much smaller than that seen in the current experiment [0.44 log_{10} units (Ref. 17) vs. 1.3 log_{10} units], suggesting that other factors were also involved.

Given that quinine and “bitterness” in general are rarely experienced by laboratory rats on a normal diet, it is possible that quinine avoidance behavior is subject to the effects of learning. Perhaps in order for input from the CT to maintain normal quinine avoidance behavior (see Ref. 33), synapses between central gustatory neurons and CT axons must be strengthened by simultaneous activity from the GL or its projections, in a Hebbian fashion. This could explain why naive rats with GL transection show modest decrements in quinine avoidance while rats with prior quinine experience show little to no change in performance after the GL is severed (17, 33). In the future it would be instructive to retrain the GL in animals that have undergone regeneration of both the CT and GL [i.e., the CT(R) + GL(R) group] and retest them for quinine avoidance to examine whether the regeneratated CT could then maintain normal performance in the absence of the GL after postregeneration quinine experience.

Overall, subjects did not appear to experience general impairments in licking with surgery that could account for changes in quinine avoidance. Analysis of water licking indicated that only the rats in the CTX + GL(R) group experienced a decrement in asymptotic licking. These rats took significantly fewer licks than the other groups to the water stimulus (Table 2) and to low concentrations of quinine, while showing normal, shamlke licking at concentrations of ≥1 mM. Chorda tympani transections in previous studies have also occasionally resulted in lick impairments (30, 33). Nevertheless, the severe attenuation of quinine avoidance exhibited by the CTX + GLX and CT(R) + GLX groups cannot be explained by a postsurgical reduction in licking at low concentrations.

It is also notable that while combined transection of the CT and GL produced a significant drop in quinine avoidance, regeneration of these nerves returned performance to presurgical levels despite a loss of ~20% of the lingual taste buds. Experiments to date have demonstrated that when a transected gustatory nerve is allowed to regenerate, taste-guided behavior returns to normal despite the reappearance of less than the normal complement of taste buds (see Refs. 13, 15, 16, 36). Until this study, however, no experiment had assessed taste-related performance after regeneration of more than one gustatory nerve. It is quite remarkable that the gustatory system of the rat is able to fully recover from such a massive injury especially given some of the reported anatomic changes in the NST (1, 9, 40). Although reinnervation of the gustatory epithelium and subsequent taste bud regeneration is necessary for behavioral recovery, it is not known whether compensatory processes in the central nervous system might also contribute.

Comparisons between the CT(R) + GL(R) group and groups in which only one nerve was allowed to regenerate failed to reveal significant differences in the number of taste pores for each reinnervated papillary field. This suggests that regeneration was independent for each nerve, and regeneration of one nerve did not appear to influence regeneration of the other.

In summary, this experiment illustrates the remarkable ability of the gustatory system of the rat to make a full functional recovery from complete gustatory deafferentation of the tongue, as assessed by a brief-access quinine avoidance paradigm. Interestingly, virtually no recovery was observed when both the CT and GL were transected and only the CT was allowed to regenerate despite the fact that GLX alone was without substantial effect in a prior test (33). Whether this result is due to changes in the peripheral or central gustatory system or both remains unclear, but the salient behavioral outcomes described here help provide a functional context for further studies aimed at defining the anatomic and physiological consequences of gustatory nerve transection and regeneration.

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