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Combined Glossopharyngeal and Chorda Tympani Nerve Transection Elevates Quinine Detection Thresholds in Rats (*Rattus norvegicus*)

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Using a conditioned shock avoidance procedure, behavioral quinine hydrochloride thresholds were measured before and after glossopharyngeal (GLX), chorda tympani (CTX), or combined glossopharyngeal and chorda tympani (GLX + CTX) transection, as well as after sham surgery. In Experiment 1, thresholds in the sham, CTX, and GLX rats (*Rattus norvegicus*) either improved (lowered) or remained the same after surgery. In Experiment 2, GLX + CTX caused a pronounced 1.5 $\log_{10}$ unit increase in presurgically measured thresholds. Neither the glossopharyngeal nor the chorda tympani nerve is necessary for normal sensitivity to low quinine concentrations provided the other is intact. When both of these nerves are transected, however, the remaining afferent input is not sufficient to maintain normal detection performance.

Quinine, an alkaloid that tastes bitter to humans, is strongly avoided by rats at millimolar concentrations. Estimates of the detection threshold for quinine hydrochloride measured behaviorally in rats are in the micromolar range (e.g., 1.2×10^{-2} mM, Koh & Teitelbaum, 1961; 5.1×10^{-3} mM, Thaw & Smith, 1994; 3×10^{-3} mM, Shaber, Brent, & Rumsey, 1970).

Quinine stimulates taste buds throughout the oral cavity, but electrophysiological studies in rats suggest that the posterior tongue taste buds convey the most specific quinine signal via the glossopharyngeal (GL) nerve (Boudreau, Do, Sivakumar, Oravec, & Rodriguez, 1987; Frank, 1991). The GL contains a large population of narrowly tuned fibers that are responsive to quinine but relatively unresponsive to sodium chloride, hydrochloric acid, and sucrose (Frank, 1991). In contrast, quinine-responsive fibers in the chorda tympani (CT) nerve are more broadly tuned, responding maximally to salts and acids (Frank, Contreras, & Hettinger, 1983; Ogawa, Sato, & Yamashita, 1968). Single units in the hamster superior laryngeal branch of the vagus nerve (SLV) respond relatively poorly to quinine and in a nonselective manner (Dickman & Smith, 1988; Smith & Hanamori, 1991). Whole nerve records of the rat greater superficial petrosal nerve (GSP) suggest that palatal taste buds are most responsive to sucrose, whereas quinine gives a far less robust response (Nejad, 1986). A single fiber analysis of this nerve has not yet been conducted, however, so it is unknown whether quinine-responsive units in the GSP are broadly or narrowly tuned.

Transection of the GL appears to be relatively benign with respect to modifying behavioral responses to quinine in freely ingesting rats. For example, GL transection did not alter quinine avoidance behavior in two-bottle preference tests (Akaike, Hiji, & Yamada, 1965; Grill, Schwartz, & Travers, 1992). This lack of an effect cannot easily be attributed to postingestive factors because GL transection failed to alter quinine drinking behavior in brief-exposure taste tests as well. For example, Yamamoto and Asai (1986) found that bilateral GL transection did not affect licking behavior of water-deprived rats to 0.5 mM quinine in 20-s taste trials. Using a more expansive array of quinine concentrations (0.003–3.0 mM), St. John, Garcea, and Spector (1994) demonstrated that GL transection did not alter the normal concentration-dependent decrease in licking during 10-s trials in water-deprived rats.

The fact that GL transection does not cause a pronounced alteration in spout licking to quinine is paradoxical in light of the existence of narrowly tuned quinine-responsive fibers in this nerve (Frank, 1991). One possible explanation is that the broadly tuned fibers of the CT and SLV, as well as the information carried by the GSP, may be sufficient to signal the presence of an unconditionally aversive gustatory stimulus. The narrowly tuned quinine-responsive units of the GL may be more critical in the gustatory discrimination of quinine from other stimuli (cf. St. John et al., 1994). A second possibility is that a GL-transected rat might be competent in responding to clearly suprathreshold concentrations of quinine but show a deficit at low concentrations. In previous studies of unconditioned licking behavior of water-deprived rats to an array of suprathreshold quinine concen-

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trations (e.g., St. John et al., 1994), effects of nerve section on sensitivity to perithreshold concentrations might have been obscured by the rat's drive to rehydrate. To directly test the hypothesis that either GL or CT transection may affect sensitivity in the perithreshold domain, we examined in the present study the effect of transecting the CT and GL, alone or in combination, on behaviorally assessed quinine detection thresholds in rats.

Experiment 1

We conducted the first experiment to determine whether quinine detection thresholds changed after GL or CT transection.

Method

Subjects. Twenty-one naive male Sprague-Dawley (Charles River Breeders; Wilmington, MA) rats (*Rattus norvegicus*) that weighed 346–414 g at the start of the experiment served as subjects. The rats were housed individually in hanging wire mesh cages in a room where temperature, humidity, and light cycle (lights on 6 a.m.–6 p.m.) were automatically controlled. All manipulations were performed during the light phase. The rats always had access to Purina Rat Chow (5001; Ralston-Purina, St. Louis, MO) in the home cage. Distilled water was also available, but was removed approximately 24 hr before the first behavioral session of the week and was replaced at the completion of the last session of the week. In most cases, behavioral testing occurred 6 days a week.

Apparatus. The rats were tested in a computer controlled gustometer (see Spector, Andrews-Labenski, & Letterio, 1990). The primary advantage of the gustometer was that it allowed taste stimuli to be delivered in brief trials while immediate responses (licks) were recorded and stored on computer disk for later analysis. The computer monitored licking behavior via a contact circuit that passed <50 nA current through the rat. Taste stimuli were contained in 12 pressurized fluid reservoirs, and fluid delivery was controlled by miniature solenoid valves that opened briefly to deposit ~5 μl of solution per lick. Taste trials were 5–10 s long depending on the phase of the experiment. Following a taste trial, the spout was rotated out of the rat's reach and rinsed with distilled water over a drainage funnel. This cleaning procedure required ~6 s.

Training. Training occurred in two phases, spout training and avoidance training. During spout training, the only fluid available was distilled water. For 2 days, training sessions were 30 min long, during which each lick delivered a drop of water. Over the next 6 days, water was delivered in brief access trials (10 s on the first 2 days, 5 s for the remainder of the experiment). The duration of the first session was 40 min. The sessions for the final days of spout training and for the remainder of the experiment were 50 min.

For the first 3 days, the rat had to lick the spout twice to initiate a trial. Over the last 3 days, a variable ratio schedule was introduced, so that by the final day (and for the remainder of the experiment) a rat had to lick a dry drinking spout between 11 and 30 times at an interlick interval less than 250 ms before fluid was delivered. This requirement ensured that the rat was actively engaged in a burst of licking and was presumably attending to the stimulus.

A time-out punishment was introduced on the 6th day of spout training. If a rat did not lick the spout at least once during the latter 3 s of the 5-s trial (avoidance period), then it received a time-out. During a time-out, the spout was withdrawn and the background white noise and house lights were turned off for 15 s. The time-out duration was increased to 30 s on the last day of spout training.

Quinine hydrochloride, made daily from reagent grade chemicals (Sigma Chemical, St. Louis, MO), was introduced during avoidance training. The purpose of this phase was to train the rats to suppress licking to quinine and maintain licking to distilled water during the avoidance period of the trial. Test trials were always preceded by a water rinse trial. A test trial consisted of a 5-s presentation of either distilled water, 0.3, 0.1, or 0.047 mM quinine. The order of test trials was randomized in blocks of six such that each block consisted of three distilled water trials and one trial at each of the three quinine concentrations. If the rat failed to suppress licking during the avoidance period when the stimulus was quinine, it received a 500-ms footshock at the end of the trial. Shock levels were titrated individually for each rat (0.15–0.37 mA). If the rat did suppress licking to quinine, it avoided the shock (i.e., a hit). The rat continued to receive a 30-s time-out if it suppressed licking to water (i.e., a false alarm).

Because some rats encountered problems avoiding the lowest concentration of quinine, 0.047 mM quinine was removed from the training stimulus array on Day 5 and 1.0 mM quinine was added on Day 14. By Day 16, all rats were clearly suppressing licking to quinine except for 1 rat that was removed from the experiment.

Presurgical testing. Threshold testing occurred for up to 27 days for the remaining 20 rats. A modified method of constant stimuli was used in which the stimulus array for each rat gradually included lower concentrations each day based on previous performance. A detectability score (DS) was derived to quantify performance:

$$DS = \frac{(W - Q_x)}{W},$$

where Q_x represents the mean licks during the avoidance period for concentration x of quinine, and W represents the mean licks on water control trials during sessions on which concentration x was tested. The DS theoretically ranges from 0 (no difference in licking to quinine and water) to 1 (no licks to quinine during the avoidance period).

The stimulus array always included, at most, four concentrations of quinine, two of which were clearly suprathreshold (one DS > 0.8, one DS > 0.5; calculated over the preceding three sessions). In general, the next lowest concentration (one-third common log steps) was added to a rat's stimulus array if the following conditions were met: (a) the rat averaged more than six licks to water during the avoidance period, (b) the rat had a DS of at least 0.8 to the highest quinine concentration, and (c) the rat had a DS of at least 0.25 to the lowest quinine concentration. The first two conditions were included to monitor whether the rat had extinguished on the training conditions. If the rat did not meet the first two conditions, then the lowest concentration in the stimulus array was removed and a higher concentration substituted until performance recovered. If the first two conditions were met but the last criterion was not for 5 consecutive days, then behavioral testing was terminated and the rat was given free access to distilled water on the home cage until all rats finished this phase.

With the exception of 3 subjects, the rats received surgery 3 to 6 days after the final rat finished presurgical testing. One rat stopped taking trials after Day 7 of testing and was removed from the experiment. In addition, it was not possible to obtain a presurgical threshold for 2 other rats in the time allowed and these rats were likewise disqualified.

Surgery. The surgical procedures used have been described in detail elsewhere (St. John et al., 1994). All rats were anesthetized

with an intraperitoneal injection of ketamine hydrochloride (86 mg/kg) and xylazine hydrochloride (13 mg/kg), and they were prophylactically treated with penicillin (30,000 units, sc; Duo-Pen, G. C. Hanford Manufacturing Co., Syracuse, NY) the day before surgery. The CT was transected in the middle ear following removal of the tympanic membrane and ossicles (CTX). The GL was transected in the ventral neck following retraction of the sublingual and submaxillary salivary glands, sternohyoideus, omohyoideus, and anterior digastricus muscles (GLX). The sham rats had the tympanic membrane punctured and the GL exposed. One rat died following the injection of anesthesia, and 1 sham rat died the day after surgery, leaving a sample size of 5 rats per group.

Postsurgical testing. The rats were given 7 days to recover. Postsurgical testing was identical to presurgical testing. The stimulus array was the same as that used in the final session of avoidance training. Lower concentrations were added as previously described. The only difference was that the rats had only 19 postsurgical test sessions; if testing continued past that point, substantial nerve regeneration could have confounded interpretation of the results (St. John, Markison, & Spector, 1995).

Water test. It was important to verify that the rats were using the chemical nature of the stimuli to discriminate quinine from water and not peculiarities associated with stimulus delivery, noise from the solenoid valves, or other possible extraneous cues. On the day following the 19th postsurgical test session, all stimulus reservoirs were filled with distilled water; half of the water reservoirs were arbitrarily associated with shock. If the rats were using extraneous cues to avoid the shock, then they should have suppressed licking to the water associated with shock and should have maintained licking to the water not associated with shock.

Histology. Immediately after the water test, the rats were deeply anesthetized with sodium pentobarbital (at least 64.8 mg/kg body weight) and perfused transcardially with saline followed by 10% buffered formalin. The lingual tissue was collected for histological evaluation. The anterior tongue was soaked in distilled water for >30 min, immersed briefly in 0.5% methylene blue, and rinsed with distilled water. The lingual epithelium was then carefully removed and pressed between two glass slides. We quantified the number of fungiform papillae and taste pores using light microscopy for sham and CTX rats to assess the efficacy of nerve section and the possibility that the CT regenerated during postsurgical behavioral testing (St. John et al., 1995).

The circumvallate papillae of sham and GLX rats were embedded in paraffin, sectioned on a rotary microtome (10 μm), mounted on glass slides, and stained with hematoxylin and eosin. The number of taste pores was quantified to assess the possibility that the GL regenerated during postsurgical behavioral testing.

Data analysis. For each rat and each concentration of quinine tested, a DS was calculated from licks averaged across all presurgical or postsurgical test trials. Because a negative DS indicates that the rat licked more to quinine than water, negative DSs were treated as zero (no discriminability). We then fit a sigmoidal curve to the DSs with the formula:

$$f(x) = \frac{1}{1 + 10^{b(x-c)}},$$

where x is the concentration given in log units, b represents the slope, and c represents the concentration at which the DS = 0.5. Threshold was defined as the value of c because this parameter effectively indicates shifts in the detectability function (Spector, Scalera, Grill, & Norgren, 1995; Spector, Schwartz, & Grill, 1990).

Because presurgical thresholds varied, we compared the postsurgical change in threshold across rats (rather than compared absolute thresholds after surgery). The change in threshold for each rat was represented as the difference between the postsurgical and presurgical values of c (i.e., the threshold in $\log_{10}$ units). A two-tailed t test was conducted on the mean change for each group to determine whether the threshold change differed significantly from zero (i.e., from no change). For the water test, the mean number of avoidance period licks to the water stimuli associated with shock was compared with that for the water not associated with shock for each rat using an independent samples t test. The statistical rejection criterion (i.e., alpha) was set at the conventional .05 level.

Results

Histology. One rat (Rat 17) in the CTX group had an unusually high percentage of fungiform papillae with taste pores (24.2%). This occurred because there was substantial regeneration on one side of the tongue (47.9% of fungiform papillae on the right side of the tongue contained a taste pore vs. only 4.7% on the left side). Consequently, Rat 17 was removed from the data analysis.[1] The remainder of the CTX rats had less than 11.3%. In contrast, all sham rats had more than 95.7%.

Two GLX rats showed some evidence of regeneration (Rat 3 had 59 taste pores; Rat 14 had 103 taste pores), but the number of pores was still clearly far below the mean number in control tissue (393 ± 21.8). A third rat in this group had 12 pores in the circumvallate papilla, and the other rats had zero pores.

Detection threshold. Before surgery, DSs for all rats increased as a function of concentration (controls, Figure 1; CTX, Figure 2; GLX, Figure 3). After surgery, controls tended to have lower detection thresholds, reflected in the leftward shift of the concentration-response function for each individual rat (Figure 1). There was little evidence that CTX altered the detectability function (Figure 2). Likewise, the postsurgical concentration-response functions for GLX rats were nearly identical to the presurgical functions (Figure 3).

The mean change in the common log of the threshold (before surgery minus after surgery) was not significantly different from zero in the CTX [0.09 $\log_{10}$ units; $t(3) = 0.88$, *ns*] and GLX groups [−0.16 $\log_{10}$ units; $t(4) = 0.86$, *ns;* see Figure 4]. If the 2 rats with partial regeneration are removed, the GLX group actually had slightly lower thresholds after surgery than before surgery [(change = −0.43 $\log_{10}$ units; $t(2) = 6.08$, $p < .05$]. As seen in Figure 1, there was an unexpected significant change in threshold for the control

[1] It is interesting to note that postsurgical thresholds were obtained for all rats except Rat 17. This rat did not show a DS less than or equal to 0.5 across the concentrations tested (including the concentration identified as the presurgical threshold). Because the scores were also distributed in a biphasic manner, a sigmoidal curve could not be fit to the data, and a threshold value could not be obtained. There was no evidence from the water test that this rat was using extraneous cues to guide its performance. Whether or not this atypical pattern of results was related to the unilateral taste bud regeneration is unknown. It should be noted that removing this rat from the analysis does not contradict the principal finding that CTX does not elevate quinine thresholds (i.e., if anything, this rat appeared to perform better at lower concentrations after surgery).

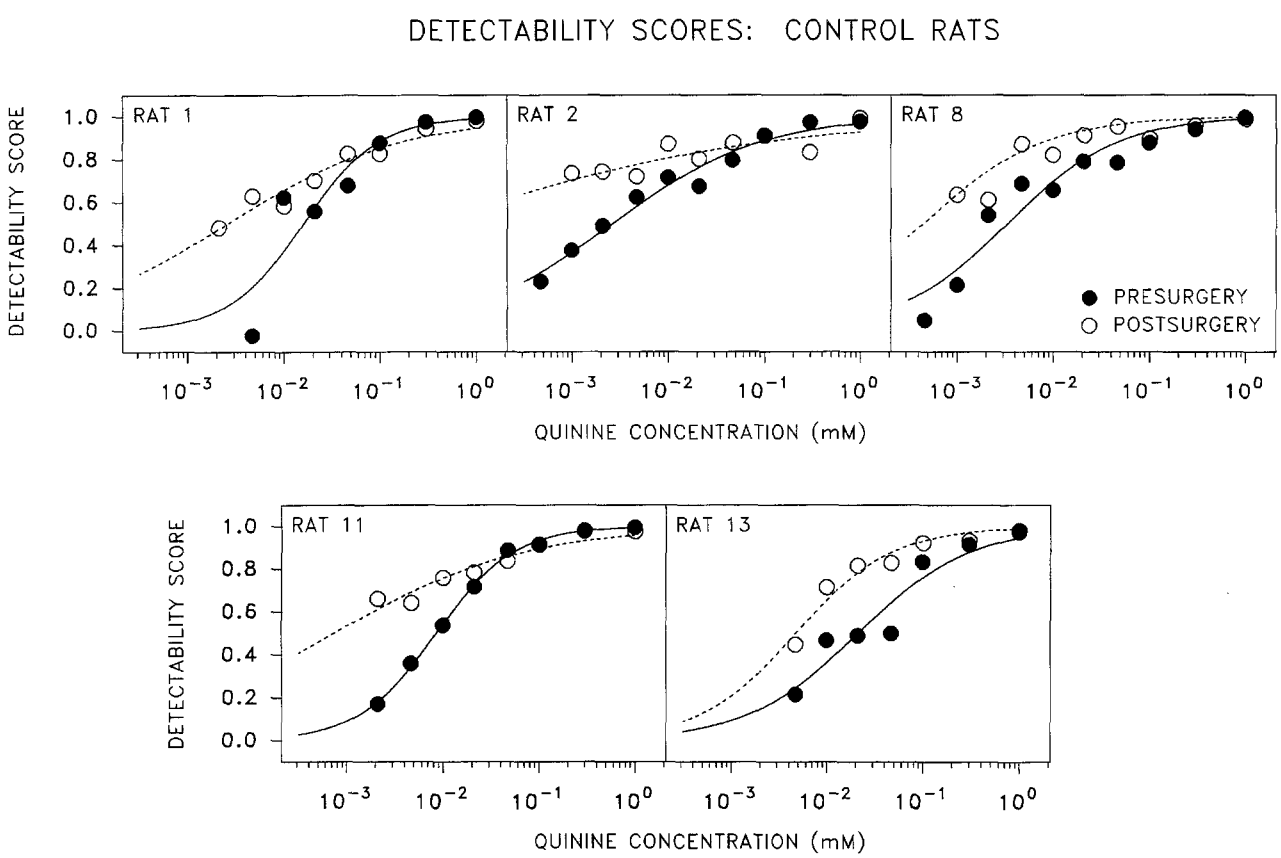

Figure 1. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each control rat both before (solid circles) and after (open circles) surgery. A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x is concentration in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

group [−1.06 $\log_{10}$ units; $t(4) = 4.63$, $p < .01$; Figure 4]. All of the rats in this group showed lower thresholds (better sensitivity) postsurgically.

Water test. There was no significant difference in the number of licks taken to the water associated with shock relative to the water not associated with shock for any rat. Thus, these rats required the presence of a chemical cue (i.e., quinine) to perform the discrimination.

Experiment 2

The first experiment demonstrated that transection of either the CT or the GL does not substantially raise quinine thresholds. If the extralingual gustatory fields supply an ultrasensitive signal for quinine, or if that input is likewise equal and redundant to the lingual input, then detection thresholds should remain unchanged after combined transection of the GL and CT. In contrast, if lingual taste receptors do contribute to quinine sensitivity, then their removal should produce a noticeable rise in thresholds.

Previously, St. John et al. (1994) reported that unconditioned lick rate in 10-s trials to a range of quinine concentrations was unaffected by either GL or CT transection, but the combined transection caused a rightward shift in the concentration-response function over one order of magnitude. One possible interpretation of those results is that the apparent decrease in quinine sensitivity seen was secondary to an elevation in the quinine detection threshold. An equally plausible interpretation is that combined GL and CT transection altered the perceived hedonic quality of quinine without raising the detection threshold. In Experiment 2, we measured quinine detection thresholds before and after combined GL and CT transection to distinguish between these two hypotheses.

Method

Subjects. Twenty-one male Sprague-Dawley rats (Charles River Breeders; Wilmington, MA) that weighed 281–357 g served as subjects. They were housed and treated the same as in Experiment 1 except where noted otherwise.

Training. The spout training phase was identical to Experiment 1. In avoidance training, we used the concentrations that we switched to starting on Day 14 in the first study (0.1, 0.3, and 1.0 mM). All rats achieved stable performance by Day 10. Shock values that were titrated during avoidance training (0.09–0.33 mA)

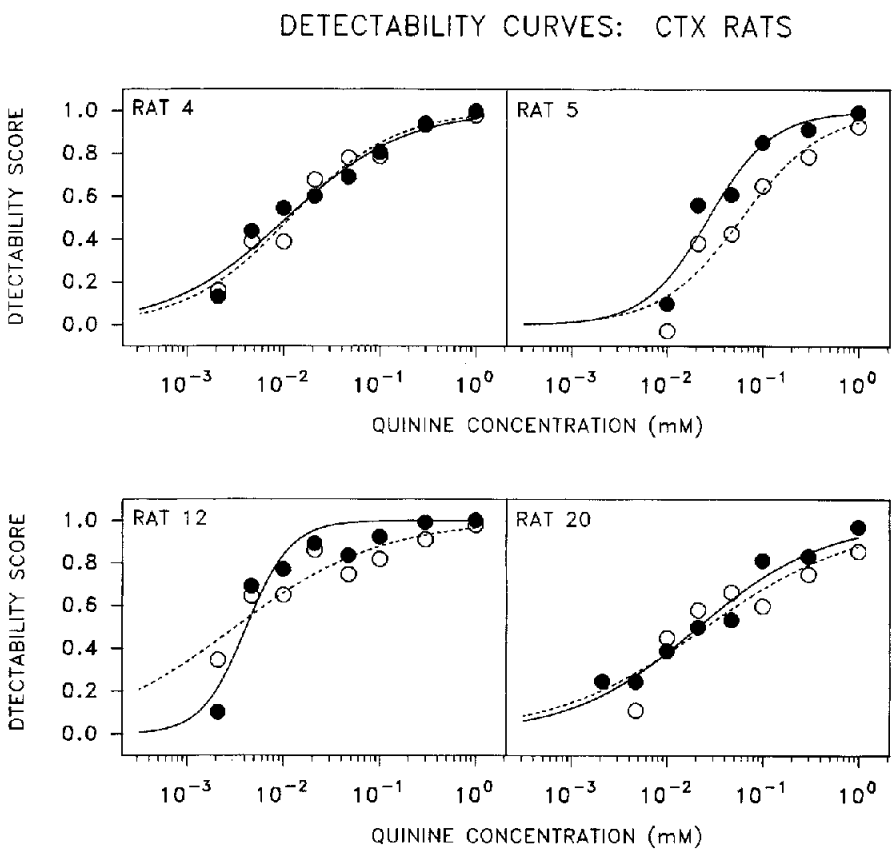

Figure 2. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each rat with bilateral chorda tympani transection (CTX) both before (solid circles) and after (open circles) surgery. A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x is concentration in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

remained constant throughout the experiment, except where noted otherwise.

Presurgical testing. One methodological difficulty in the first experiment was that some thresholds for control rats had to be slightly extrapolated after surgery. Because regeneration can occur within about 28 days of gustatory nerve transection (St. John et al., 1995), we were limited in the postsurgical duration of the test. To address that issue, we made some minor changes in the selection of the stimulus array in Experiment 2 to reach thresholds more rapidly.

In the second experiment concentrations were selected on the basis of a DS computed over a two-session period rather than a three-session period. Thus, a rat in this experiment could move to lower concentrations every 2 days as long as the criteria listed in Experiment 1 were met. These criteria remained the same, except that rats had to show five licks to water in the avoidance period rather than six licks. Finally, with the exception of the first presurgical threshold assessment (see below), quinine concentrations were added in one-half common logarithmic steps rather than one-third steps.

A second change in the basic design was prompted by the postsurgical performance of the control group in Experiment 1. In an effort to assess the stability of thresholds with repeated testing, we conducted two (not one) presurgical threshold tests and the rats were screened on this basis. Only rats that had a difference of less than 0.6 $\log_{10}$ units between the two baseline tests were selected to continue in the experiment.

Surgery. Five rats received sham surgery, identical to Experiment 1. Seven rats had the GL and CT exposed as previously described, but in this experiment we cauterized the nerves (GLX + CTX) using a hand-held cautery unit with an elongated tip (Roboz, Rockville, MD). The ossicles were not removed during the CT surgery as they were in Experiment 1. One rat in each group died shortly after surgery. All rats were allowed 7 days to recover except Rats 4 and 8, which regained body weight somewhat more slowly than other rats in the GLX + CTX group and were allowed 9 days for recovery.

Postsurgical testing. The first postsurgical test was identical to the second presurgical test. All but 1 rat in each group was then given a second postsurgical test. Because 3 rats in each group appeared to habituate somewhat to the shock, these rats were tested in the second postsurgical test with the shock level retitrated and elevated by 0.05–0.11 mA. Two rats in the GLX + CTX group were tested in the second postsurgical threshold test with the shock levels unchanged (because these rats were exhibiting adequate responsiveness to the shock). The remaining rats could not be tested twice because of time constraints. The purpose of the second postsurgical test was (a) to test rats with weak responses to the

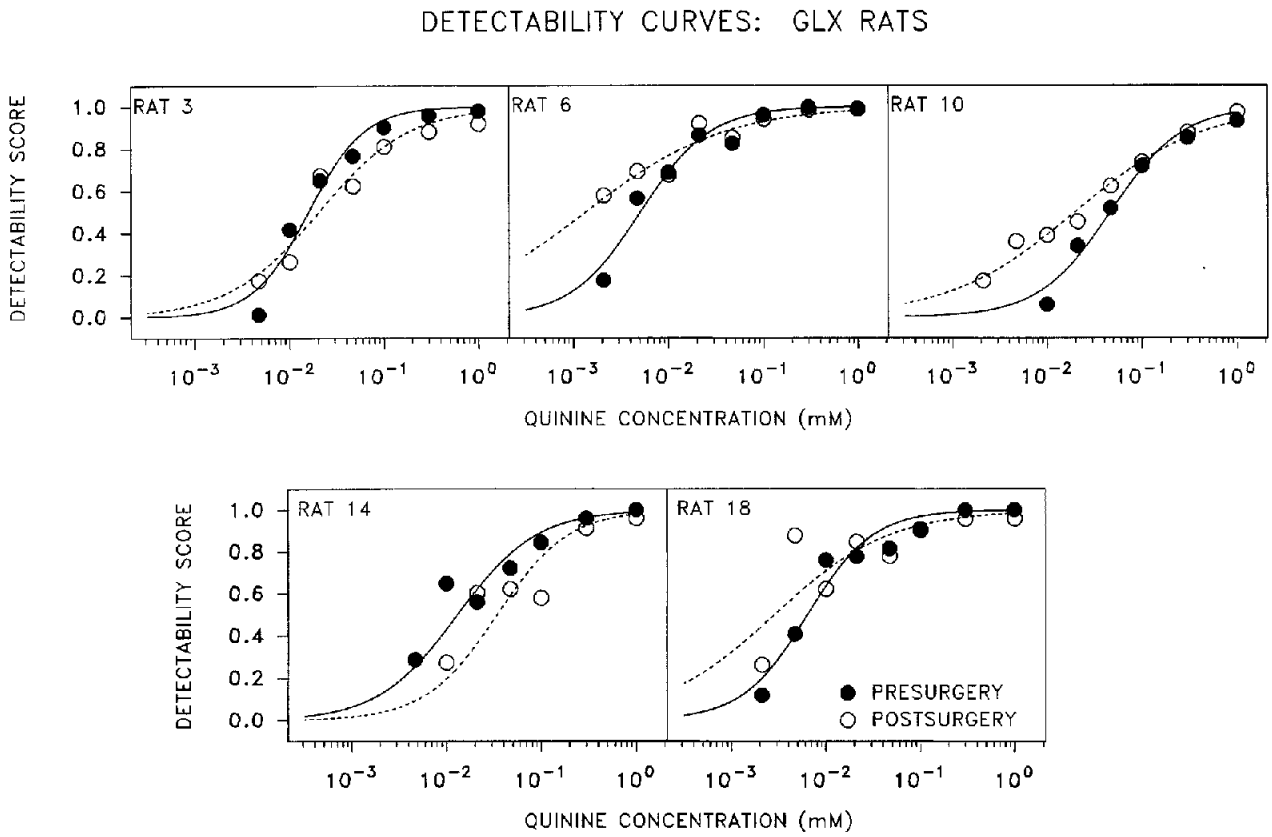

Figure 3. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each rat with bilateral glossopharyngeal nerve transection (GLX) both before (solid circles) and after (open circles) surgery. A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x is concentration in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

shock at a more appropriate shock level and (b) to further test the stability of threshold measurements over time. In addition, because some rats in the GLX + CTX group did not suppress licking to quinine even at the highest concentration (1.0 mM), 3.0 mM was included in the second test.

Water test. A subset of rats (n = 7) was given the water control test.

Data analysis. The data were analyzed in the same manner as in Experiment 1.

Results

Histology. All rats in the GLX + CTX group had no taste pores in the circumvallate papilla and had fewer than 15% of the fungiform papillae containing a taste pore. Control rats all had greater than 97% of fungiform papillae containing a taste pore.

Detection threshold. In contrast to Experiment 1, controls in this study generally had slightly higher thresholds after sham surgery, as indicated by the rightward shift in the concentration-response function (Figure 5). The mean change in log threshold was 0.69 log units, $t(3) = 5.43$, $p < .02$. This moderate threshold increase contrasts sharply with the considerable threshold elevation of rats in the GLX + CTX group (Figure 6). In 5 out of 6 GLX and CTX rats, the threshold increased by a factor of 30 to 100. In fact, for most rats in this group, the threshold had to be extrapolated to concentrations greater than those tested (note that the DS was less than 0.5 even at 1.0 mM for several rats in Figure 6).

Curve fits were not obtained for Rat 4, Rat 6, and Rat 8. In the case of Rat 6 and Rat 8, the data were not distributed in a sigmoidal fashion (Figure 6). For Rat 8, the DS was below 0.5 at all concentrations tested, whereas Rat 6 had a low DS (0.48) at a midrange concentration but higher DSs at lower concentrations. Because the concentrations tested for Rat 8 were 2 $\log_{10}$ units above the presurgical threshold, and because this rat did not have a DS of 0.5 at any tested concentration, this rat's postsurgical threshold was estimated to be at least 2 log units greater than the presurgical threshold. We estimated the threshold for Rat 6 to be the same as the presurgical value because this rat's performance was good at most concentrations near the presurgical threshold. In the case of Rat 4, the DS was virtually zero at all concentrations tested. Because the highest tested concen-

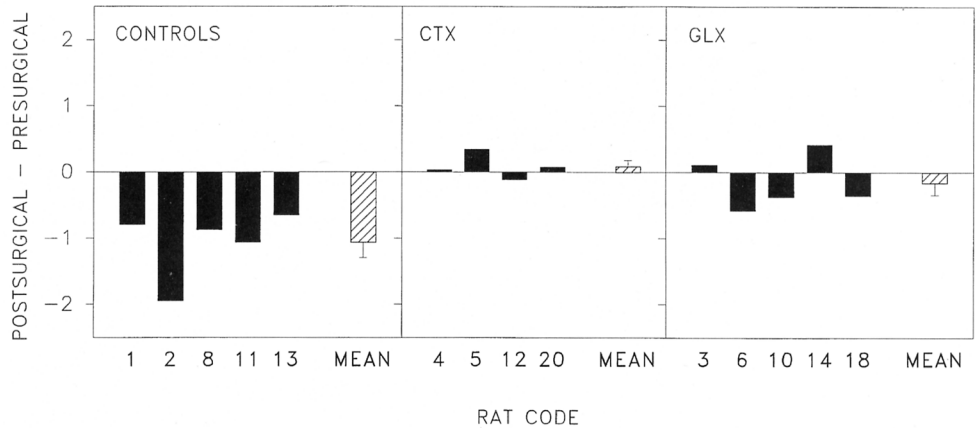

Figure 4. The change in threshold (solid bars) represented as the common log of the postsurgical threshold minus the common log of the presurgical threshold for each rat receiving sham surgery (CONTROLS, left), chorda tympani transection (CTX, middle), and glossopharyngeal transection (GLX, right). The group means (± *SE*) are also given (hatched bars). Positive values indicate elevated thresholds after surgery and represent a decrease in sensitivity. Negative values indicate lower thresholds after surgery.

tration was more than 1 log unit greater than the presurgical threshold, this rat's change in $\log_{10}$ threshold was conservatively estimated at 1.5. Given these approximations, the mean threshold change in this group was 1.42 $\log_{10}$ units ± 0.30, $t(5) = 4.76$, $p < .01$. A statistical test confirmed that these rats were more affected than controls, $t(8) = 2.38$, $p < .05$.

All but 1 rat in each group was given a second postsurgical threshold test. Three rats in each group were tested with elevated shock levels in order to test the possibility that the increased thresholds seen in the first postsurgical test were a result of habituation to the shock (Figure 7). Relative to the final presurgical test, the controls with raised shock values had slightly lower thresholds postsurgically [−0.23 $\log_{10}$ units shift, $t(2) = 2.23$, *ns*, whereas the GLX + CTX group remained substantially compromised [1.57 $\log_{10}$ unit shift, $t(2) = 6.16$, $p < .05$]. The 3 control rats either had no difference in threshold between the second test and the presurgical test or actually had a significantly lower threshold postsurgically (Rat 7). In contrast, Rat 4 and Rat 10 of the GLX + CTX group had significantly higher thresholds even after the shock levels were raised. In addition, although a statistical test could not be performed for Rat 8 because of the nonsigmoidal nature of the postsurgical data, it is clear this rat also remained substantially compromised.

The 2 rats in the GLX + CTX group that were tested a second time with unchanged shock levels showed divergent results. One rat still showed a large increase in the quinine detection threshold (1.76 $\log_{10}$ units). The other rat appeared to benefit from further experience, but there was not time to test this rat at lower concentrations (see *Method*). Thus, it is unclear how much lower the threshold on the second test would have been.

The results are summarized in Figure 8, which gives the change in $\log_{10}$ threshold based on each rat's final postsurgical threshold test. In general, there was no consistent change in threshold in control rats, but in 5 of 6 rats with GLX + CTX, there was a large, 13-fold to 100-fold increase in threshold.

Water test. As in Experiment 1, no rat from the subset tested showed any evidence of being able to suppress licking to the water that signaled shock relative to the water that did not. In the one significant case ($p < .05$), the rat actually appeared to suppress licking to the water that did not signal shock. This statistical outcome was probably due to chance; if the test had incorporated a Bonferonni control for experiment-wise error, the result would have been nonsignificant.

General Discussion

Combined GL and CT transection caused a pronounced elevation in behaviorally assessed quinine hydrochloride detection thresholds. In contrast, transection of either nerve alone resulted in virtually no change in threshold. A similar profile of results was found in studies of unconditioned licking to suprathreshold concentrations of quinine in brief access taste trials (St. John et al., 1994; Yamamoto & Asai, 1986). These results indicate that neither the GL nor the CT is necessary to maintain normal sensitivity to quinine, but the combined input of the two nerves is necessary.

Effects of GL or CT Section on Quinine Detection Thresholds (Experiment 1)

Neither GLX nor CTX resulted in a significant change in postsurgical performance to low quinine concentrations relative to the presurgical assessment. However, all control rats had lower thresholds after sham surgery. Because quinine thresholds in rats do not improve with age (Thaw,

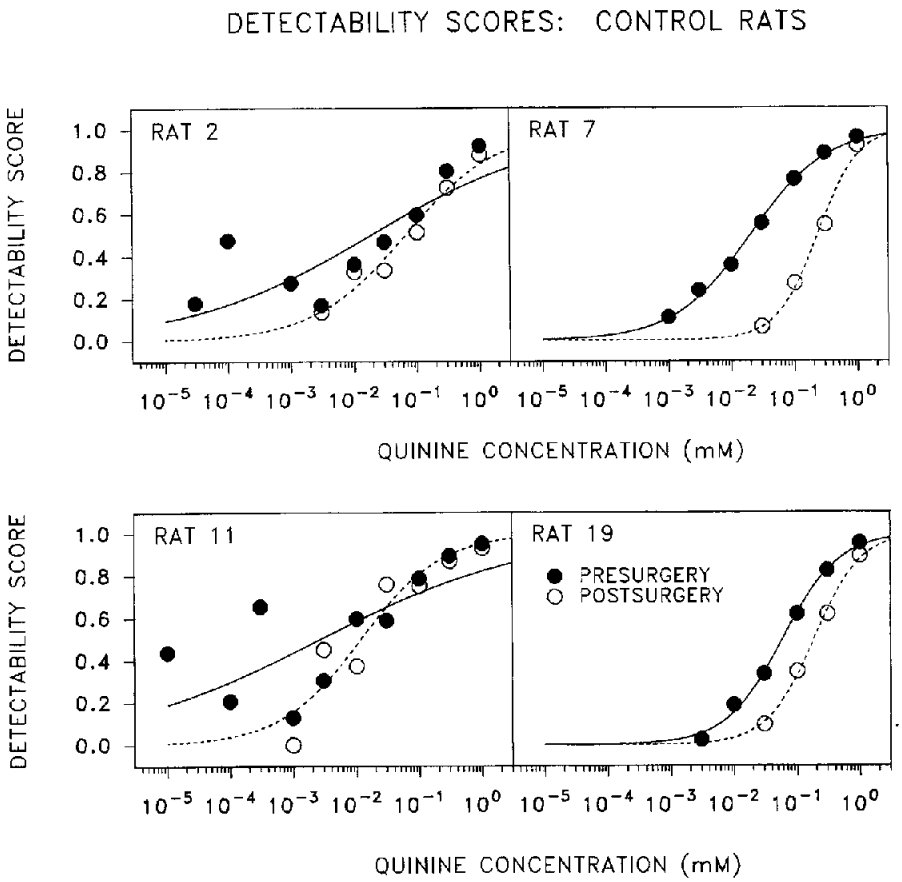

Figure 5. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each control rat in the final presurgical test (solid circles) and the first postsurgical test (open circles). A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x concentration is in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

1996), either some effect of sham surgery or continued experience in the task produced enhanced quinine sensitivity. The sham surgical procedure was not substantially different from the nerve transection surgery, however, so this explanation seems unlikely. It seems more likely that continued experience with the experimental paradigm allowed control animals to display greater sensitivity to quinine, perhaps due to continued familiarity with the training contingencies, up-regulation of peripheral quinine receptors, or strengthening of central synapses processing the peripheral signal representing quinine. Beyond whatever mechanism underlies the enhanced sensitivity to quinine, it appears that transection of either the CT or the GL prevents nerve-transected rats from displaying these same benefits of experience.

In light of these speculations, the following conclusions can be drawn. First, CTX or GLX does not result in the elevation of presurgically measured quinine detection thresholds. Second, CTX and GLX moderately attenuate the experience-guided enhancement in quinine sensitivity seen in control rats in the present experimental design. Third, beyond these moderate effects of nerve section, the CT and GL do not appear to differentially contribute to behavioral responses to very low concentrations of quinine. This was unexpected because of the differential response properties of GL and CT afferents in electrophysiological studies (Boudreau et al., 1987; Frank, 1991; Frank et al., 1983; Ogawa et al., 1968). Apparently, the rat's sensitivity to quinine is maintained following removal of either those broadly tuned CT fibers or those more narrowly tuned GL fibers that are responsive to this test stimulus.

Effects of Combined GL and CT Section on Quinine Detection Thresholds (Experiment 2)

In contrast to the effects of single nerve section, GLX + CTX had a pronounced effect on the quinine detection threshold, elevating it, in some cases, by more than 1.5 $\log_{10}$ units. Lingual gustatory input is, therefore, necessary for the maintenance of normal sensitivity to low concentrations of quinine in rats. Following removal of the GL and CT, rats must rely on input from the palatal and pharyngeal taste buds

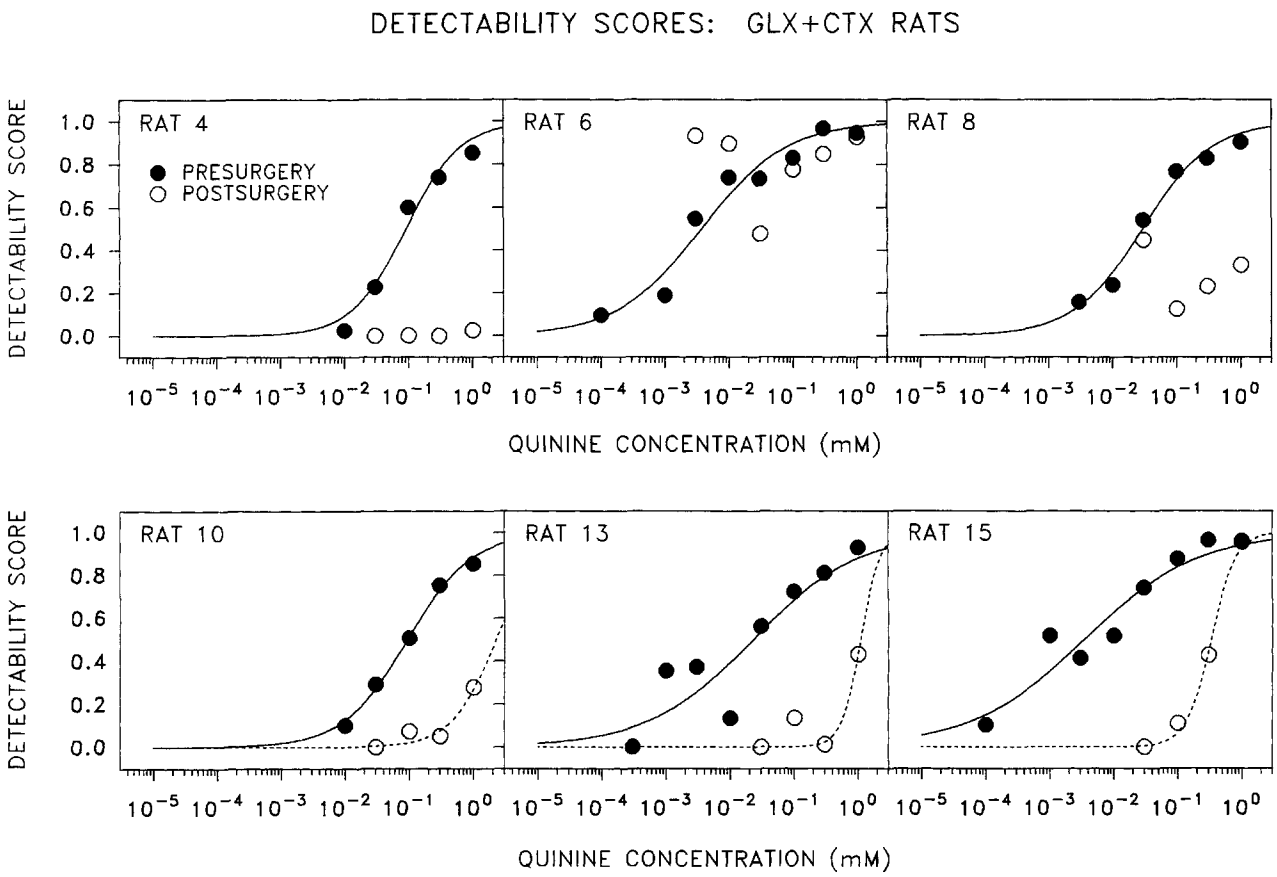

Figure 6. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each rat with combined chorda tympani and glossopharyngeal nerve section (GLX + CTX) in the final presurgical test (solid circles) and the first postsurgical test (open circles). A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x concentration is in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

innervated by the GSP and SLV, respectively. Although rats do not appear to be aguesic to quinine following denervation of the lingual taste buds (St. John et al., 1994), their sensitivity to quinine is greatly compromised.

In addition to taste bud denervation, combined GL and CT transection also partially denervates the salivary glands. Although GLX denervates von Ebner's gland, it also removes most of the taste buds these glands subserve in the circumvallate and foliate papillae (Gurkan & Bradley, 1987). The major source of innervation of the rat parotid glands is a branch of the auriculo-temporal nerve (Greene, 1963), but the CT partially innervates the sublingual and submaxillary salivary glands (Young & Van Lennep, 1978). These latter glands, however, are also innervated by lingual nerve fibers (Hellekant & Kasahara, 1973). No effect of removing the sublingual and submaxillary salivary glands was seen on quinine licking behavior in brief access trials (St. John et al., 1994). The partial denervation of these glands was also insufficient to elevate quinine detection thresholds in Experiment 1 (i.e., the CTX group). Whether or not the altered salivary environment contributed to the threshold elevation seen in Experiment 2 must be considered as a possibility until explicit tests are conducted.

In Experiment 1, control rats displayed lower thresholds after sham surgery. This experience effect was not replicated in the second experiment. There are at least three explanations for this. First, the procedure was not identical in the two studies; in fact, procedural changes were made specifically to minimize the experience effect. Second, the rats were given two presurgical threshold determinations and were screened on the basis of threshold stability. It is possible that the rats most likely to show an enhancement with further experience were removed from the study. Third, many of the control and nerve-sectioned rats appeared to habituate to the shock. Shock reinforces lick suppression to quinine in this paradigm, so that rats that begin to habituate to the shock may be able to detect quinine but not suppress their licking if the shock is not sufficiently aversive. When the shock level was raised for the 3 sham rats that appeared to habituate to the shock, the thresholds returned to the presurgical level (or slightly lower).

Three rats in the GLX + CTX group that also appeared to

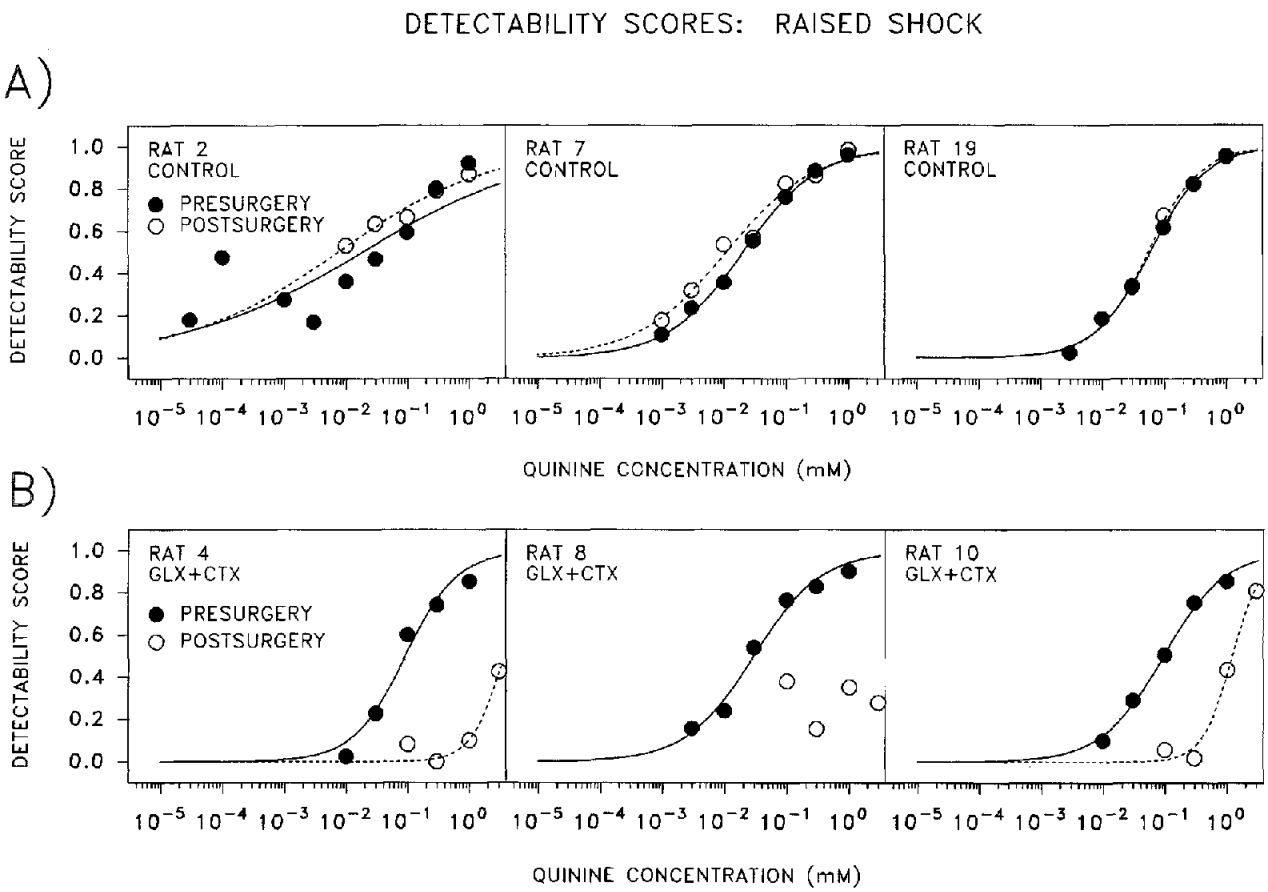

Figure 7. The detectability scores (0 = no lick suppression to quinine relative to water; 1 = complete lick suppression to quinine) as a function of quinine concentration for each rat that had raised shock values in the second postsurgical test (open circles) relative to the presurgical test (solid circles). A: Rats with control surgery (CONTROL); B: Rats with combined chorda tympani and glossopharyngeal nerve section (GLX + CTX). A least squares sigmoidal curve was fit to the data with the equation $f(x) = 1/(1 + 10^{b(x-c)})$, where x is concentration in common log units, b is the slope, and c is the operationally defined threshold concentration of detectability score = 0.5; the best-fit curve is plotted for presurgical (solid line) and postsurgical (dashed line) data.

habituate to the shock had raised shock levels in the second postsurgical test. Whereas this manipulation returned control thresholds to the presurgical value, the thresholds for the nerve-sectioned rats remained substantially elevated. It appears, then, that any deficits seen in the control group in the first postsurgical test can be attributed to an inadequate shock level, but the more substantial deficits in the experimental group can be attributed solely to the nerve section.

Combined GL and CT nerve section denervates approximately 80% of the total number of taste buds in rats, including all of the taste buds on the tongue (Miller, 1977). Such a dramatic insult to the peripheral gustatory system apparently does not render rats aguesic to quinine, as demonstrated by the residual responsivity to 3 mM quinine in some rats in this study and by the avoidance of high quinine concentrations in a study of unconditioned licking behavior to suprathreshold quinine concentrations (St. John et al., 1994). Surprisingly, others have shown minor effects of GLX + CTX on taste-guided responses to other taste stimuli. For example, GLX + CTX had no significant effect (Grill & Schwartz, 1992; Vance, 1967) or only a moderate effect (Pfaffmann, 1952) on NaCl preference. A recent preliminary report demonstrated no effect of GLX + CTX on unconditioned licking behavior of water-deprived rats to an array of NaCl concentrations (0.03–1 M) in 10-s taste trials (Cauthon, Garcea, & Spector, 1994). In addition, GLX + CTX had only minor effects on unconditioned licking to an array of sucrose or maltose concentrations, whereas combined transection of the GSP and CT (which denervates about 30% of the total taste bud population) caused a pronounced change in responsiveness to these two sugars (Spector, Redman, and Garcea, 1996). Thus, although the elevation in quinine thresholds after such major gustatory denervation should not be unexpected, this effect is not seen in all behavioral paradigms or with all taste stimuli.

Finally, there was evidence that 1 rat was not impaired by GLX + CTX, and another rat may have showed improvement during the second postsurgical test. Therefore, al-

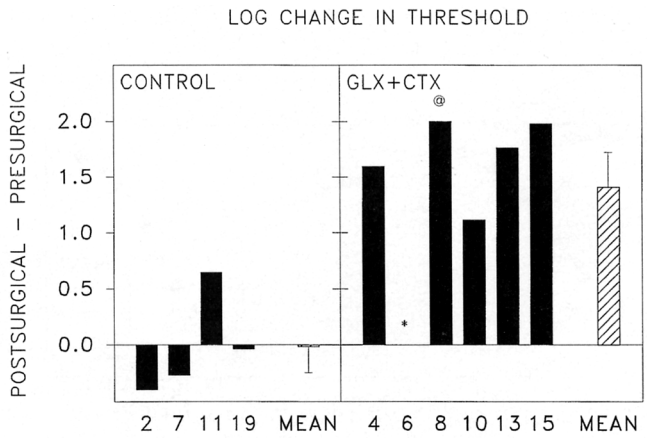

Figure 8. The change in threshold (solid bars) represented as the common log of the final postsurgical threshold minus the common log of the final presurgical threshold for each rat receiving sham surgery (CONTROL, left) and combined chorda tympani and glossopharyngeal nerve transection (GLX + CTX, right). A curve could not be fit to the data for Rat 6 because of the nonsigmoidal nature of the concentration-response function (see Figure 3). Because this rat appeared to be performing well, we conservatively set the threshold change for this rat at zero (*). Similarly, the data for Rat 8 were nonsigmoidal (see Figure 3). Because the detectability score for this rat was less than 0.5 (i.e., the operationally defined threshold) at the highest tested concentration, which was 2 log units higher than the presurgical threshold for this rat, the threshold change for this rat was conservatively set at 2 log units (@). The data for Rat 15 comes from the first postsurgical test as there was insufficient time to test the curve fully in the second test (because the nerves may regenerate in approximately 28 days; St. John et al., 1995). The group means (± *SE*) are also given (hatched bars) and include all rats in the group. Positive values indicate elevated thresholds after surgery and represent a decrease in sensitivity. Negative values indicate lower thresholds after surgery.

though there is clearly a substantial effect on quinine thresholds in most rats with GLX + CTX, some rats may be less affected. Presumably, this results from individual variation in the palatal and lingual distribution of high affinity quinine transduction mechanisms, but this remains to be explicitly tested.

The Role of the GL and CT in Quinine Perception in Rats

The GL contains an electrophysiologically defined population of taste fibers that are narrowly tuned to respond to quinine and possibly other substances considered bitter by humans (Boudreau et al., 1987; Frank, 1991). Behavioral experiments, however, do not completely support the conclusion that the GL plays a predominant role in quinine taste sensation. Single nerve cuts (GL or CT section) have minimal effects on most taste-guided behavior to quinine, whereas combined transection of the two nerves has a major effect.

For example, GL section does not alter preference–avoidance functions for quinine (Akaike et al., 1965; Grill et al., 1992). In contrast, Vance (1967) found that combined transection of the GL, CT, and pharyngeal branch of the vagus caused approximately a 1 log unit shift in the preference–avoidance function. St. John et al. (1994) reported similar results in rats with GLX + CTX in an experiment designed to measure immediate unconditioned responses to an array of randomly presented quinine concentrations. Although GLX was shown to cause a substantial reduction in aversive taste reactivity to intraorally infused quinine, GLX + CTX completely eliminated the concentration-dependent increase in aversive responses across the concentration range tested (0.03–3 mM; Grill et al., 1992; Grill and Schwartz, 1992). The present experiment was the first to examine effects of these manipulations on liminal sensitivity, and it demonstrated that combined transection raised quinine detection thresholds 1.5 $\log_{10}$ units, whereas single cuts had virtually no effect.

In all of these studies, the effect of combined nerve transection was far greater than the sum of the effects of the individual neurotomies. The balance of the behavioral data including our study suggests that the GL, CT, and possibly the GSP are somewhat redundant in their contribution to taste-guided responses to suprathreshold and perithreshold quinine concentrations (Grill et al., 1992; St. John et al., 1994; Vance, 1967; Yamamoto & Asai, 1986). The nonadditive effects of GLX + CTX imply that quinine-evoked signals in the GL and CT converge centrally (St. John et al., 1994; Yamamoto & Asai, 1986).

Despite the behavioral evidence suggesting that quinine signals from all taste bud receptor fields contribute to quinine-evoked behavioral responses, there is some behavioral evidence supporting a special role for the GL. First, GLX (but not CTX) does substantially reduce the number of aversive oromotor responses evoked by intraorally infused quinine (Grill et al., 1992; Travers, Grill, & Norgren, 1987). The taste reactivity paradigm focuses on the consummatory phase of ingestion, which presumably has a large reflexive component. It has been argued that the GL may play a predominant (but not exclusive) role in mediating aversive oromotor responses organized in the caudal brainstem (Travers et al., 1987).

In addition, two preliminary reports demonstrated a moderate effect of GLX on appetitive spout-licking studies with quinine. First, Spector, St. John, and Klumpp (1995) reported that GLX increased the 45-min intake of 0.2 mM quinine hydrochloride in rats following 24-hr water deprivation. Control rats and rats with CTX reduced the size of bursts of licking 0.2 mM quinine by 90% relative to a water intake test, whereas rats with GLX had 60% smaller bursts relative to water. Second, in an unconditioned-licking study that was identical to the St. John et al. (1994) study except that rats received quinine for the first time after surgery, GLX shifted the concentration-response function about 0.5 log unit relative to controls (Markison, St. John, & Spector, in press). In these two experiments, as in the taste reactivity studies, quinine was novel to the GLX rats. Thus, the effects of GLX may be more severe if the rats have not had presurgical experience with the stimulus.

Absolute Thesholds for Quinine

Although there are a variety of interpretive difficulties in comparing detection thresholds across studies (Morrison,

1974), it should be noted that the quinine thresholds measured in the present experiment are within the range of those arrived at by other investigators using quinine hydrochloride (Koh & Teitelbaum, 1961; Shaber et al., 1970; Thaw & Smith, 1994) or quinine sulfate (Morrison & Norrison, 1966). The geometric mean absolute threshold for all 14 rats in the presurgical test of Experiment 1 was 1.053×10^{-2} mM; for all 10 rats in the second presurgical test of Experiment 2 the mean threshold was 1.835×10^{-2} mM. These values closely match those of the other studies cited above.

Conclusions and Implications

These experiments, in the context of other nerve-transection studies testing quinine, suggest that combined GL and CT transection causes a substantial but not complete loss of quinine taste sensitivity. Single nerve transections do not cause substantial deficits in quinine taste sensitivity, although moderate deficits may become apparent in some behavioral procedures. Because the effect of combined transection is greater than the sum of the effect of GLX or CTX alone, the quinine-evoked input of the GL and CT is somewhat redundant and may converge centrally.

Finally, the fact that CT units responding to quinine also respond to salts and acids suggests a role for the narrowly tuned quinine units of the GL in sensory discrimination. That is, whereas GL transection does not remove all of the quinine responsive fibers in the peripheral gustatory system of the rat, it may substantially reduce the number of relatively quinine-specific fibers. The appetitively driven behavioral studies listed so far have merely measured the rat's preference for quinine or the ability to discriminate quinine from water. Neither task necessarily requires that the rat identify the stimulus as quinine. The possibility that GL transection results in deficits of stimulus generalization and discrimination could be explored using taste aversion generalization paradigms (eg., Nowlis, Frank, & Pfaffmann, 1980) and operant discrimination procedures (Spector & Grill, 1992), respectively.

References

Akaike, N., Hiji, Y., & Yamada, K. (1965). Taste preference and aversion in rats following denervation of the chorda tympani and the IXth nerve. *Kumamoto Medical Journal, 18,* 108–109.

Boudreau, J. C., Do, L. T., Sivakumar, L., Oravec, J., & Rodriguez, C. A. (1987). Taste systems of the petrosal ganglion. *Chemical Senses, 12,* 437–458.

Cauthon, R., Garcea, M., & Spector, A. C. (1994). Taste-guided unconditioned licking to suprathreshold sodium chloride concentrations is unaffected by selective lingual denervation [abstract]. *Chemical Senses, 19,* 452.

Dickman, J. D., & Smith, D. V. (1988). Response properties of fibers in the hamster superior laryngeal nerve. *Brain Research, 450,* 25–38.

Frank, M. E. (1991). Taste-responsive neurons of the glossopharyngeal nerve of the rat. *Journal of Neurophysiology, 65,* 1452–1463.

Frank, M. E., Contreras, R. J., & Hettinger, T. P. (1983). Nerve fibers sensitive to ionic taste stimuli in the chorda tympani of the rat. *Journal of Neurophysiology, 50,* 941–960.

Greene, E. C. (1963). *The anatomy of the rat.* Philadelphia: Hafner Press.

Grill, H. J., & Schwartz, G. J. (1992). The contribution of gustatory nerve input to oral motor behavior and intake-based preference. II. Effects of combined chorda tympani and glossopharyngeal nerve section in the rat. *Brain Research, 573,* 105–113.

Grill, H. J., Schwartz, G. J., & Travers, J. B. (1992). The contribution of gustatory nerve input to oral motor behavior and intake-based preference. I. Effects of chorda tympani or glossopharyngeal nerve section in the rat. *Brain Research, 573,* 95–104.

Gurkan, S., & Bradley, R. M. (1987). Autonomic control of von Ebner's lingual salivary glands and implications for taste sensation. *Brain Research, 419,* 287–293.

Hellekant, G., & Kasahara, Y. (1973). Secretory fibers in the trigeminal part of the lingual nerve to the mandibular salivary gland of the rat. *Acta Physiologica Scandinavica, 89,* 198–207.

Koh, S. D., & Teitelbaum, P. (1961). Absolute behavioral taste thresholds in the rat. *Journal of Comparative and Physiological Psychology, 54,* 223–229.

Marksion, S., St. John, S. J., & Spector, A. C. (in press). Unconditioned licking of quinine is increased by glossopharyngeal nerve transection in rats without presurgical stimulus exposure. *Chemical Senses.*

Miller, I. J. (1977). Gustatory receptors of the palate. In Y. Katsuki, M. Sato, S. Takagi, & Y. Oomura (Eds.), *Food intake and chemical senses* (pp. 173–186). Tokyo: University of Tokyo Press.

Morrison, G. R. (1974). Taste thresholds, taste sensitivity, and the effects of adrenalectomy in rats. *Chemical Senses and Flavor, 1,* 77–88.

Morrison, G. R., & Norrison, W. (1966). Taste detection in the rat. *Journal of Comparative and Physiological Psychology, 20,* 208–217.

Nejad, M. S. (1986). The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. *Chemical Senses, 11,* 283–293.

Nowlis, G. H., Frank, M. E., & Pfaffmann, C. (1980). Specificity of acquired aversions to taste qualities in hamsters and rats. *Journal of Comparative and Physiological Psychology, 94,* 932–942.

Ogawa, H., Sato, M., & Yamashita, S. (1968). Multiple sensitivity of chorda tympani fibres of the rat and hamster to gustatory and thermal stimuli. *Journal of Physiology, 199,* 223–240.

Pfaffmann, C. (1952). Taste preference and aversion following lingual deafferentation. *Journal of Comparative and Physiological Psychology, 45,* 393–400.

Shaber, G. S., Brent, R. L., & Rumsey, J. A. (1970). Conditioned suppression taste thresholds in the rat. *Journal of Comparative and Physiological Psychology, 73,* 193–201.

Smith, D. V., & Hanamori, T. (1991). Organization of gustatory sensitivities in hamster superior laryngeal nerve fibers. *Journal of Neurophysiology, 65,* 1098–1114.

Spector, A. C., Andrews-Labenski, J., & Letterio, F. C. (1990). A new gustometer for psychophysical taste testing in the rat. *Physiology and Behavior, 47,* 795–803.

Spector, A. C., & Grill, H. J. (1992). Salt taste discrimination after bilateral section of the chorda tympani nerve or the glossopharyngeal nerve. *American Journal of Physiology, 263,* R169–R176.

Spector, A. C., Redman, R., & Garcea, M. (1996). The consequences of gustatory nerve transection on taste-guided licking of sucrose and maltose in the rat. *Behavioral Neuroscience, 110,* 1096–1109.

Spector, A. C., Scalera, G., Grill, H. J., & Norgren, R. (1995).

Gustatory detection thresholds after parabrachial nuclei lesions in rats. *Behavioral Neuroscience, 109,* 939–954.

Spector, A. C., Schwartz, G. J., & Grill, H. J. (1990). Chemospecific deficits in taste detection after selective gustatory deafferentation in rats. *American Journal of Physiology, 258,* R820–R828.

Spector, A. C., St. John, S. J., & Klumpp, P. A. (1995). Patterns of quinine licking in rats: Contribution of lingual taste buds [abstract]. *Obesity Research, 3,* 384s.

St. John, S. J., Garcea, M., & Spector, A. C. (1994). Combined, but not single, gustatory nerve transection substantially alters taste-guided licking behavior to quinine in rats. *Behavioral Neuroscience, 108,* 131–140.

St. John, S. J., Markison, S., & Spector, A. C. (1995). Salt discriminability is related to number of regenerated taste buds after chorda tympani nerve transection in rats. *American Journal of Physiology, 269,* R141–R153.

St. John, S. J., & Spector, A. C. (1995a). Chorda tympani or glossopharyngeal nerve section does not alter quinine detection thresholds in rats [abstract]. *Chemical Senses, 20,* 785.

St. John, S. J., & Spector, A. C. (1995b). Combined glossopharyngeal and chorda tympani nerve section profoundly raises quinine detection thresholds in rats [abstract]. *Obesity Research, 3,* 328s.

Thaw, A. K. (1996). Changes in taste threshold over the life span of the Sprague-Dawley rat. *Chemical Senses, 21,* 189–193.

Thaw, A. K., & Smith, J. C. (1994). Conditioned suppression as a method of detecting taste thresholds in the rat. *Chemical Senses, 17,* 211–223.

Travers, J. B., Grill, H. J., & Norgren, R. (1987). The effects of glossopharyngeal and chorda tympani nerve cuts on the ingestion and rejection of sapid stimuli: An electromyographic analysis in the rat. *Behavioural Brain Research, 25,* 233–246.

Vance, W. B. (1967). Hypogeusia and taste preference behavior in the rat. *Life Sciences, 6,* 743–748.

Yamamoto, T., & Asai, K. (1986). Effects of gustatory deafferentation on ingestion of taste solutions as seen by licking behavior in rats. *Physiology and Behavior, 37,* 299–305.

Young, J. A., & Van Lennep, E. W. (1978). *The morphology of the salivary glands.* London: Academic Press.

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