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The Contribution of Taste Bud Populations to Bitter Avoidance in Mouse Strains Differentially Sensitive to Sucrose Octa-acetate and Quinine

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Abstract

Mice of the SWR/J (SW) strain avoid orally delivered sucrose octa-acetate (SOA), whereas the mice of the C3HeB/FeJ (C3) strain are insensitive to SOA. Mice of both strains and of a congenic strain (C3.SW) that shares more than 99% of the C3 genome, were tested in a taste-salient brief-access taste test for responses to SOA and quinine hydrochloride, before and after transection of the glossopharyngeal or chorda tympani nerve, or sham surgery. Prior to surgery, congenic SOA tasters (C3.SW^T) were phenotypically identical to the SW strain in avoidance of SOA, but showed a greater reduction in sensitivity after nerve transection. For quinine avoidance, which is thought to be a polygenic trait, SW mice showed the greatest sensitivity to quinine, C3 the least and C3.SW^T mice were different from both parental strains, showing intermediate sensitivity. Nerve transections had only a moderate effect on quinine sensitivity, suggesting that both anterior and posterior taste bud fields contribute to behavioral quinine avoidance. These findings are discussed with regard to the distribution in the oral cavity of putative taste receptors for quinine and SOA and the peripheral organization of bitter taste.

Key words: bitter avoidance, mouse, quinine, sucrose octa-acetate, taste bud populations

Introduction

The primary taste sensation described by humans as 'bitter' performs a crucial function in ingestion: the detection of potentially toxic foodstuffs. Chemicals that are perceived as aversive are heterogeneous in structure, suggesting that no single mechanism can account for bitter taste transduction (Spielman *et al.*, 1992). An important mechanism for bitter taste transduction is the activation of metabotropic receptors coupled to gustducin, a G-protein that initiates taste receptor depolarization via a phospholipase C (PLCβ2) second messenger cascade (Wong *et al.*, 1996; Ming *et al.*, 1999; Zhang *et al.*, 2003). The ability to respond to the diversity of potentially toxic chemicals is afforded, in part, by a diversity of metabotropic receptors. Candidate bitter receptors, dubbed T2Rs, are products of an estimated 34 functional mouse Tas2r genes predominantly located in several clusters on chromosome 6 (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000; Shi *et al.*, 2003; Zhang *et al.*, 2003).

The T2Rs are not distributed uniformly in the oral cavity. For example, Adler *et al.* (2000) report that, in rats, whereas all vallate and foliate taste buds contain T2R-expressing taste receptor cells, <10% of taste buds in the fungiform papillae contain T2R-expressing cells (as assessed by *in situ* hybridization). Equivalent quantitative data are unavailable in mice, but indications are that these regional differences are similar in rats and mice (Nelson *et al.*, 2001). These data are consistent with electrophysiological observations: namely, that whereas the glossopharyngeal nerve (GL), which innervates vallate and foliate taste buds, responds well to compounds humans describe as bitter, the chorda tympani nerve (CT), which innervates predominantly fungiform taste buds, generally responds poorly to those compounds (e.g. Pfaffmann, 1955; Oakley, 1967; Ogawa *et al.*, 1968; Ninomiya *et al.*, 1982, 1984; Frank *et al.*, 1983; Boudreau *et al.*, 1985, 1987; Shingai and Beidler, 1985; Nejad, 1986; Frank, 1991; Inoue *et al.*, 2001; Danilova and Hellekant, 2003).

Surprisingly, then, behavioral responses to aversive compounds are often maintained following transection of the GL. For example, bilateral transection of the GL does not alter responses to quinine in two-bottle preference tests (Akaike *et al.*, 1965; Grill *et al.*, 1992) or in brief-access tests in which the contribution of post-ingestive effects are minimized (Yamamoto and Asai, 1986; St. John *et al.*, 1994).

Responses to cetylpyridinium chloride, a salt that appears to taste like quinine to rats, are likewise unaffected by GL transection (Hallagan *et al.*, 2003). Transection of the GL does not disrupt the discrimination of quinine from KCl (St. John and Spector, 1998), nor does it elevate presurgically measured quinine detection thresholds (St. John and Spector, 1996). Just as surprising, transecting the CT in addition to the GL causes pronounced impairments in each of these tasks (Pfaffmann, 1952; Vance, 1967; Jacquin, 1983; Yamamoto and Asai, 1986; Grill and Schwartz, 1992; St. John *et al.*, 1994; St. John and Spector, 1996, 1998; Hallagan *et al.*, 2003), suggesting that any weak signal generated in anterior tongue taste buds might be critical in many behavioral responses to aversive substances.

To date, behavioral responses to aversive substances following peripheral nerve injury have been assessed primarily in rats. Less is known about the mouse. Mice constitute an especially promising model for the study of bitter taste, given robust differences among inbred strains in sensitivity to bitter-tasting stimuli (Ninomiya *et al.*, 1984; Shingai and Beidler, 1985; Whitney and Harder, 1994; Nelson *et al.*, 2003). The source of these differences are generally not well-defined, although evidence strongly suggests that polymorphisms in *Tas2r* bitter taste receptor genes play a major role (Chandrashekar *et al.*, 2000; Bachmanov *et al.*, 2001). We were interested in assessing the effects of gustatory nerve cuts on taste-guided behavior in strains of mice that differ in sensitivity to bitter stimuli. SWR/J (SW) mice display robust avoidance to a broad array of bitter stimuli, including quinine and the bitter acetylated sugar sucrose octaacetate (SOA). In contrast, C3HeB/FeJ (C3) mice are quinine 'non-tasters' and display avoidance of SOA at a point that is three log steps higher than SW mice. We also took advantage of the existence of C3.SW-*Soa*^{a} congenic taster mice, which possess a small region of chromosome 6 from the SW (donor) strain transposed to a 99% C3 bitter-insensitive background. Significantly, this fragment contains loci implicated in quinine and SOA sensitivity (termed *Qui* and *Soa*, respectively); these loci are tightly linked to a cluster of *Tas2r* genes (Bachmanov *et al.*, 2001) and therefore likely represent particular *Tas2rs*, although specific candidates affecting quinine or SOA sensitivity have not been identified.

Materials and methods

Subjects

Eighty-seven mice from two inbred and one congenic strain were tested under three different experimental conditions: Before and after bilateral transection of the CT (CTX), the GL (GLX), or sham surgery (CON). Members of the C3 and SW inbred strains were purchased from Jackson Laboratories (Bar Harbor, ME) or were laboratory bred (litters from progenitors purchased from Jackson Laboratories). Congenic C3.SW mice were laboratory bred (see below). All mice were naïve at the beginning of the experiment and were tested as adults. At least 1 week prior to the experiment, mice were separated into individual plastic shoebox cages in a colony room where temperature and lighting was automatically controlled. Food (Harlan Teklad, 7012) and water were available *ad libitum*, except where noted in Procedure. Group sizes, sex information and initial body weights for all strains are given in Table 1.

C3.SW^T Congenic Mice

SW mice are noted for gustatory sensitivity to a variety of aversive compounds including SOA and quinine, whereas C3 mice do not avoid many of the same chemicals on the basis of taste. Using these strains as progenitors, Boughter and Whitney (1995, 1998) developed a congenic strain to isolate the single locus (termed *Soa*) with a major effect on SOA sensitivity. Through a process of phenotypic selection (two-bottle preference tests, SOA versus water) across >11 generations, the dominant Soa^a (taster) allele was introgressed from the SW donor strain to the C3 genomic background. These mice have been maintained by continual backcrossing of phenotypic tasters to C3 inbred mice—any given backcross generation produces 50% tasters and 50% non-tasters.

Because we were interested in congenic tasters in this experiment (henceforth, C3.SW^T), congenic animals were first screened by means of a two-bottle preference test

Table 1 Numbers of mice in each condition

Strain	Surgery	Sex	Weight (g)	*n*	Total *n*
C3	CON	M	25.6 ± 0.59	9	
		F	20.0 ± 0.00	2	11
	CTX	M	24.3 ± 0.75	4	
		F	20.3 ± 1.03	4	8
	GLX	M	25.4 ± 1.03	8	
		F	20.3 ± 0.67	5	13
SW	CON	M	19.9 ± 1.07	4	
		F	18.9 ± 1.83	6	10
	CTX	M	22.8 ± 1.02	5	
		F	17.3 ± 0.88	3	8
	GLX	M	20.4 ± 1.24	3	
		F	19.9 ± 0.50	5	8
C3.SW^T	CON	M	31.2 ± 1.38	9	
		F	25.0 ± 2.65	3	12
	CTX	M	28.7 ± 1.20	3	
		F	26.8 ± 2.29	4	7
	GLX	M	34.3 ± 1.47	5	
		F	27.3 ± 0.98	5	10

(Figure 1). Only mice meeting the taster requirement (drinking <15% of total fluid from the SOA bottle in two consecutive, 48 h, two-bottle preference tests between water and 0.1 mM SOA) were used in the current study. 'Screening' conducted in this fashion produces unambiguous identification of tasters and non-tasters (Boughter and Whitney, 1995, 1998; Boughter *et al.*, 2002). Because more C3.SW^T mice were identified than could be tested in this experiment at a given time, only some C3.SW^T mice screened actually served as subjects in the current study. With the exception of a consideration to balance the number of males and females in the study, which taster mice were chosen for the experiment out of those meeting the criterion during any given screening test was random.

This screening procedure took place a few days or weeks prior to the main experiment. Thus, one difference in the treatment of the parental strains and the C3.SW^T mice in the current study was that the latter had exposure to SOA prior to testing. We have previously reported that this limited preexposure to SOA has absolutely no effect on lick–concentration functions generated in the brief-access behavioral assay used in the current report (Boughter *et al.*, 2002).

Apparatus

Mice were tested daily in an automated lickometer referred to as the 'Davis Rig' (Davis MS-160; DiLog Instruments, Tallahassee, FL). The Davis Rig allows the presentation of up to 16 different taste stimuli within a single behavioral session, with the duration and order of stimulus presentation at the control of the experimenter (Rhinehart-Doty *et al.*, 1994; Smith, 2001). The test chamber consisted of a plastic rectangular cage ($30 \times 14.5 \times 18$ cm) with a wire mesh floor; an oval opening centered in the front wall allows access to taste solutions contained in leak-proof sipper tubes. Fluid access can be restricted by a computer-operated shutter.

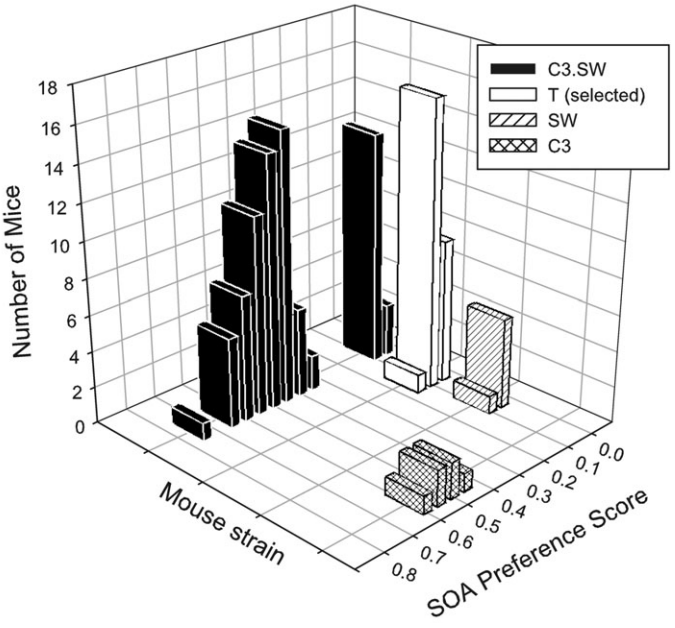

Figure 1 Frequency distributions of individual preference scores for mice screened with 0.1 mM SOA. C3.SW mice (black and white bars) are unambiguously classified as tasters (preference ratio ≤ 0.15) or non-tasters (preference ratio > 0.15). White bars represent screen data for tasters subsequently used in this study; blacks bars represent all other progeny screened, including tasters who were not used in this study. A few C3 and SW inbreds (hatching) were tested along with C3.SW progeny as a validity check. The C3 and SW mice that participated in these screening tests were not used in the brief-access experiments reported here.

Trials began with the opening of the shutter and ended 5 s after the mouse made its first lick on the drinking spout (see Procedure). Licks were counted with a high-frequency AC contact circuit. Failure to initiate a lick within 300 s also ended a trial (although such a 'zero lick trial' was ignored in analyses of lick rate as the failure to initiate licking could not be an orosensory-based behavior). In between trials, a platform upon which the stimulus tubes were mounted was driven to a new location. Although the time that the motor was activated was variable, the intertrial interval (15 s) was always constant. The session ended only after the completion of all 30 trials.

Stimuli and experimental design

Each iteration of the experiment lasted 3 weeks. During the first week, mice were habituated to the Davis Rig (two sessions) and were then tested for responses to water, SOA and quinine. SOA (0.00018, 0.001, 0.006, 0.03 and 0.18 mM) and quinine (quinine hydrochloride, 0.01, 0.03, 0.1, 0.3 and 1 mM) were obtained from Sigma (St Louis, MO) and were made fresh weekly. During the second week, mice were given surgery and during the third week, mice were re-familiarized with the apparatus and were tested postsurgically in a manner identical to the first week.

Procedure

Prior to each week of behavioral testing, water was removed from the home cage ~24 h prior to the initial Davis Rig session of the week. Tap water was returned to the home cage at the end of each week of testing and was available *ad libitum* during the surgical recovery interval.

On the first session, mice obtained a single, 20 min access to distilled water from a single drinking tube. Occasionally, mice did not learn that water was available and were retested later in the day. Most mice took at least 100 licks from the drinking spout in this first session.

On the following day, mice were familiarized with the brief-access trial structure. In our initial experiments, water was still obtained from a single drinking tube during this training session, but the shutter closed and reopened on the 15 s intertrial interval over the course of 30 training trials. The majority of mice were, however, tested with water delivered from 11 different drinking tubes, to more faithfully reflect the testing conditions.

Testing occurred over the next 3 days in three identical sessions. In each of these sessions, behavioral responses to both SOA and quinine were assessed, under the following randomized block design. There were five concentrations each of SOA and quinine and water was a sixth stimulus for each concentration series. The 30 trials were divided into five blocks of 6 trials each. A given mouse either received SOA–quinine–SOA–quinine–rinse over its five blocks, or quinine–SOA–quinine–SOA–rinse. Within an SOA block, the five concentrations of SOA and water were randomly presented. Within a quinine block, the five concentrations of quinine and water were randomly presented. The final rinse block was six consecutive presentations of water. In pilot studies, we had noted that some mice were not motivated to initiate trials during the fifth block. The final block therefore allowed the thirstiest mice to rehydrate, without compromising data interpretation (during which low lick rates could either reflect avoidance or lack of motivation to drink).

In sum, each test session provided two data points per taste concentration (for a total of six across all three sessions), four data points for water and six rinse trials that were not analyzed and served merely to rehydrate the animal.

Four to five days later, mice underwent surgery (see Surgery). Four to five days following surgery, water was again removed from the home cage and mice received five sessions identical to the presurgical sessions. After the final test, the mouse was perfused and the lingual tissue was collected to allow histological analysis of the nerve transections (see Histology).

Surgery

Mice were deeply anesthetized with 4% chloral hydrate (400 mg/kg, i.p.). For GLX, the ventral neck was shaved and prophylactically treated with iodine solution (Betadine). An incision in the skin of the neck permitted access to the GL following dissection of the fascia surrounding the sublingual and submaxillary salivary glands. The GL was visualized infereolateral to the hypoglossal nerve and was cut bilaterally with microscissors. Visualization of the GL required blunt dissection of the fascia between the sternohyoid, the omohyoid and the digastric muscles; retraction of the muscles (other than by the blunt forceps) proved unnecessary. The nerve was exposed by removing the fascia around it and was cut using microscissors. The incision was closed by suture (4-0).

For CTX, the mouse was fixed in a custom headholder that permitted access to the ear with the animal's head titled 80° away from the surgeon. One curved No. 7 microforceps was used to temporarily widen the auditory meatus to allow visualization of the structures of the middle ear and a second forceps was used to remove the tympanic membrane. Deflection of the malleus allowed visualization of the CT which was severed with the forceps. The malleus was then removed.

Surgical controls had the GL exposed but not cut. Refer to Table 1 for the number of mice in each group completing the entire experiment.

Histology

After the final day of postsurgical testing, mice were deeply anesthetized with 4% chloral hydrate and were perfused with saline and 10% w/v buffered formalin. The tongue of each mouse was removed and stored in formalin. For CTX and a subset of CON rats (n = 4/strain), the anterior tongue was soaked in distilled water for 30 min, immersed in 0.5% w/v methylene blue and then rinsed with distilled water. The epithelium was removed and pressed between two slides in order to observe the fungiform papillae under a light microscope. The percentage of fungiform papillae containing taste pores was calculated for each mouse; a low percentage of papillae with pores indicates a successful bilateral CT transection (Whitehead *et al.*, 1987; Ganchrow and Ganchrow, 1989; St. John *et al.*, 1995; Parks and Whitehead, 1998).

For GLX and CON mice, the circumvallate papilla was embedded in paraffin and sectioned on a rotary microtome (10 μm) through the extent of the papilla. Tissue sections were mounted consecutively on glass slides and were stained with hematoxylin and eosin. The slides were observed under a light microscope; the lack of taste buds in this receptor field indicates a successful bilateral GL transection.

Unfortunately, technical errors during histological preparation resulted in an irretrievable loss of posterior tongue tissue in 13 cases. Based upon the substantial evidence from the surviving tissue that taste buds do not appear 10 days after our surgical procedure, we elected to include all cases in the main data analyses. Subsidiary analyses were performed on those cases exclusively that were histologically verified (see Results).

Data analysis

Lick rate during the distilled water taste trials provides an assessment of the maximal lick rate on a mouse by mouse basis. Thus, in order to standardize for differences in lick rate, the primary dependent measure computed for each mouse (across all presurgical or all postsurgical sessions) was (average number of licks to tastant_x)/(average number of licks to water), where x is a given concentration of SOA or quinine and the average number of licks to water is derived from the water trials during the SOA and quinine blocks only (see Procedure). This taste/water ratio thus ranges from a hypothetical zero (complete avoidance) to one (no difference from water). A taste/water ratio of zero is impossible because zero lick trials (~10% of the total) were not counted in this analysis. Concentration–response functions were also fit with a two-parameter logistic function:

$$f(x) = \frac{1}{1 + (x/c)b} \quad (1)$$

where x is the concentration of SOA or quinine, c is the concentration of evoking half-maximal avoidance (i.e. a taste/water ratio of 0.5) and b is the slope. One advantage of fitting curves is to provide a single parameter (c) which is sensitive to leftward or rightward shifts in the concentration–response function as a result of surgery.

The potential role of olfaction was assessed by analysis of the latency to initiate trials. Evidence that mice differentially delayed initiating trials at higher concentrations was interpreted to mean that the mice were able to sense the identity of the proffered stimulus prior to licking. Since visual or auditory cues are unlikely in the Davis Rig, a significant effect of concentration on trial initiation would potentially implicate olfaction.

All variables were analyzed by analysis of variance (ANOVA) or dependent t-tests using the conventional statistical rejection criterion of 0.05.

Results

Histology

As mentioned in Materials and methods, a number of vallate tissue were irretrievably lost prior to verification of the nerve transections. In seven SW mice, four C3.SW^T mice and seven C3 mice, we were able to determine that no taste buds appeared in the vallate papilla following GLX.

No mouse with CTX had more than eight stained taste pores, whereas no control mouse had <43. The presence of a few stained pores following CTX is consistent with complete gustatory denervation; methylene blue stain appears to have a small 'false positive' rate (St. John *et al.*, 1995; Parks and Whitehead, 1998). In addition to dramatically reducing the number of stained taste pores, CTX is also associated with a reduction in the number of fungiform papillae on the anterior tongue and an alteration of their morphology (Oakley *et al.*, 1990, 1993; St. John *et al.*, 1995). In rats, CTX is associated with a loss of ~25% of fungiform papillae (St. John *et al.*, 1995).

Our work in mice has uncovered a potentially interesting strain difference between SW and C3 mice in the importance of CT innervation for the structural integrity of fungiform papillae (Table 2). SW mice had nearly a 60% reduction in papilla number after CTX (relative to SW controls), whereas C3 and C3.SW^T mice lost 35 and 25%, respectively. An ANOVA indicated that the three strains differed on the number of fungiform papillae seen after CTX [$F(2,20) = 14.7$, $P < 0.0005$]; a Bonferroni-corrected *post hoc* test indicated that the SW mice differed from the other two strains (P-values < 0.002), which did not themselves differ from one another ($P > 0.8$).

Strain differences in SOA and quinine responsivity

The C3 and SW inbred strains are demitasters and tasters of SOA respectively and all of the C3.SW^T mice selected for inclusion in the experiment express the SW taster phenotype.

Table 2 Anterior tongue histological results

Strain	Surgery	Pores	Papillae	% with pores
C3	CON	64.3 ± 6.4	66.3 ± 6.6	97.0 ± 1.0
	CTX	4.4 ± 0.5	43.3 ± 3.3	10.3 ± 1.0
SW	CON	62.0 ± 1.7	64.3 ± 2.5	96.6 ± 1.3
	CTX	3.0 ± 0.9	26.6 ± 1.9[a]	10.3 ± 2.5
C3.SW^T	CON	61.3 ± 6.5	63.5 ± 6.0	96.1 ± 1.5
	CTX	2.4 ± 0.5	48.0 ± 3.5	4.9 ± 0.9

[a]The strains differed in the number of papillae after CTX; a *post hoc* test indicated this group differed from the other two.

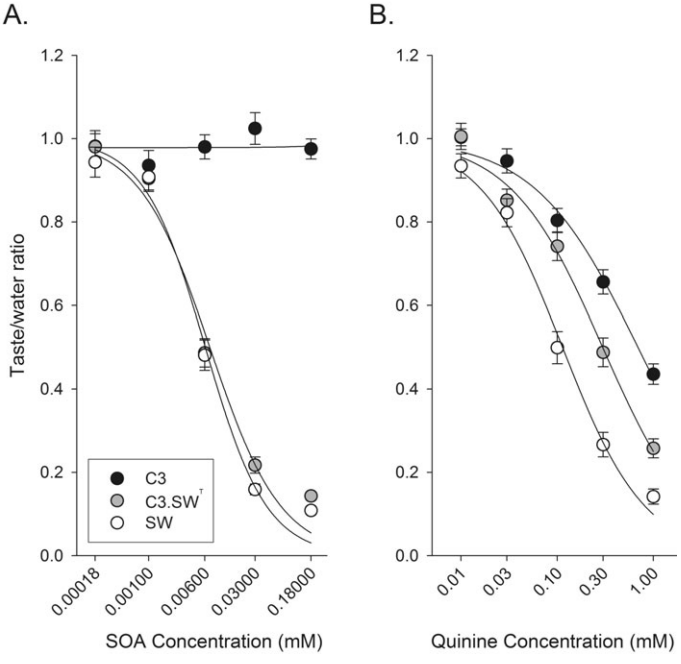

Figure 2 Strain comparison of avoidance of SOA **(A)** and quinine **(B)** in C3 (dark circles), SW (white circles) and C3.SW^T (gray circles) mice prior to surgery (i.e. surgical groups were ignored). Sigmoidal curves were fit to mean data (see text for details); a straight line was fit to C3 data in (A).

Screening was determined by a two-bottle preference test. However, as we have shown previously (Boughter *et al.*, 2002), C3, SW and C3.SW^T mice clearly exhibit either the SOA nontaster or taster phenotype in brief access taste tests as well. A strain × concentration ANOVA (summed across all members of a strain, regardless of future surgical manipulation) revealed that the three strains differed in their presurgical responses to SOA [strain, $F(2,84) = 175.8$, $P < 0.00001$]. As can be seen in Figure 2A, the SW and C3.SW^T mice avoided SOA at concentrations >0.001 mM SOA, whereas C3 mice licked all concentrations at the same rate as water. Sigmoidal curves were fit to the individual data for each mouse, which provides a single parameter—c, see equation (2)—that can be used to compare taste sensitivity across

strains (this value represents the concentration at which the lick rate is half that of water). A *t*-test on the log-transformed *c* parameter revealed that the SW and C3.SW^T mice did not differ [$t(52) = 0.17$, $P = 0.87$]. The geometric mean of the c-parameter was 0.0060 mM in the SW mice and 0.0062 mM in the C3.SW^T mice; these values are consistent with those in our recent report (Boughter *et al.*, 2002). Thus, C3.SW^T and SW mice respond identically to SOA in this paradigm.

In contrast, all mice were quinine tasters, but exhibited three distinct phenotypes (Figure 2B). A strain × concentration ANOVA indicated that the strain factor was significant [$F(2, 84) = 29.8$, $P < 0.00001$]. In order to test which strains differed from which, the logarithm of the *c*-parameter from the sigmoidal curve fits to the data for each mouse was used to compare strain sensitivity. An ANOVA revealed strain differences [$F(2, 84) = 43.2$, $P < 0.00001$] and a Tukey *post hoc* test indicated that that all three strains differed from one another (all *P*-values < 0.0001). The geometric mean of the *c*-parameter was 0.109 mM for the SW mice, 0.283 mM for the C3.SW^T mice and 0.639 mM for the C3 mice. These fairly dramatic differences in sensitivity range from about one-third of a log unit (C3.SW^T mice versus C3 mice) to three-quarters of a log unit (SW versus C3 mice).

Role of olfaction

In contrast to lick rate, there were not pronounced differences among the strains in the latency to initiate taste trials; nor did latency grow markedly with concentration presurgically (Figure 3A). When latency was subjected to statistical analysis in strain × concentration ANOVAs for each taste stimulus, however, there was statistical evidence that these factors affected latency to lick. (Water was treated as a 'zero concentration' in these analyses.) There was a main effect of SOA concentration on latency to lick [$F(5,420) = 4.49$, $P < 0.001$] as well as a strain × concentration interaction [$F(10,420) = 2.82$, $P < 0.005$]. One-way ANOVAs conducted at each concentration indicated that the strains differed significantly only at the 0.03 mM SOA concentration [$F(2,84) = 3.72$, $P < 0.05$]; Tukey *post hoc* tests indicated that the SW mice differed from the C3.SW^T mice ($P < 0.05$). This difference was probably driven by three members of the SW strain that showed unusually high median latencies at that concentration. Latency to lick can increase as a function of olfactory cues, but may also be due to other strain differences (overall activity levels, propensity to engage in exploratory or grooming behaviors, etc.). These reliable effects on latency are thus difficult to interpret.

Quinine latencies were somewhat more orderly (Figure 4, right panel). Latency grew slightly with concentration [$F(5,420) = 5.49$, $P < 0.0005$] and the strains differed [$F(2,84) = 3.16$, $P < 0.05$]. Separate ANOVAs at each concentration revealed that the strains differed at 0.03 mM quinine [$F(2,84) = 3.19$, $P < 0.05$; Tukey tests indicated that the C3 and C3.SW^T differed] and 0.1 mM quinine [$F(2,84) = 3.45$, $P < 0.05$; Tukey tests indicated that the SW and C3.SW^T differed]. Additionally, there was a trend for the strains to differ on water trials as well [$F(2,84) = 2.65$, $P = 0.076$, n.s.], suggesting that the strain difference might not have an exclusively olfactory basis.

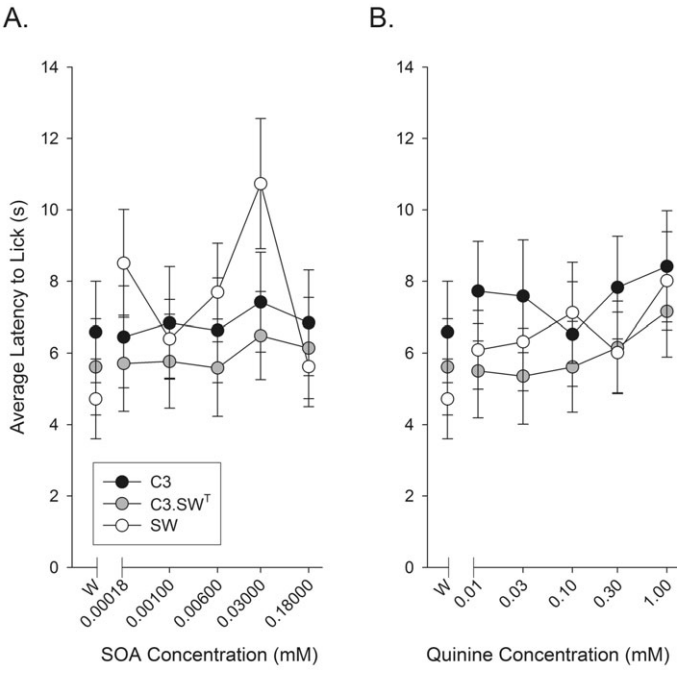

Figure 3 Strain comparison of latency to initiate taste trials of SOA **(A)** and quinine **(B)** in C3 (dark circles), SW (white circles) and C3.SW^T (gray circles) mice prior to surgery (i.e. surgical groups were ignored). Although there were not large-magnitude differences across strain or concentration for latency to initiate either SOA or quinine trials, there was statistical evidence of differences in latency for both factors (see text for details). Only presurgical trials are shown. W = water trials. Latencies are means (±SE) of the median latencies for individual mice.

The effect of nerve transection on SOA sensitivity

Mice from the C3 strain did not avoid SOA at any concentration used in our study (see Figure 2A) and were unaffected by nerve transection (Figure 4A). Nerve transection did, however, decrease sensitivity to SOA in the taster strains (Figure 4B,C). Separate test (i.e. presurgical versus postsurgical tests) × concentration ANOVAs for each strain–surgery combination indicated that GLX reduced avoidance of SOA in both SW mice [test, $F(1,7) = 14.19$, $P < 0.01$] and C3.SW^T mice [test, $F(1,9) = 36.3$, $P < 0.001$; test × concentration, $F(4,36) = 4.88$, $P < 0.01$]. Additionally, CTX had no effect on SW avoidance of SOA, but did affect C3.SW^T mice [test, $F(1,6) = 13.48$, $P < 0.02$, test × concentration, $F(4,24) = 7.36$, $P < 0.001$]. Paired *t*-tests on the logarithm of the *c*-parameter from individual curve fits confirmed these results. The SW mice averaged a 0.40 log unit shift rightward in the concentration–response functions after GLX and C3.SW^T mice averaged a 0.68 log unit shift.

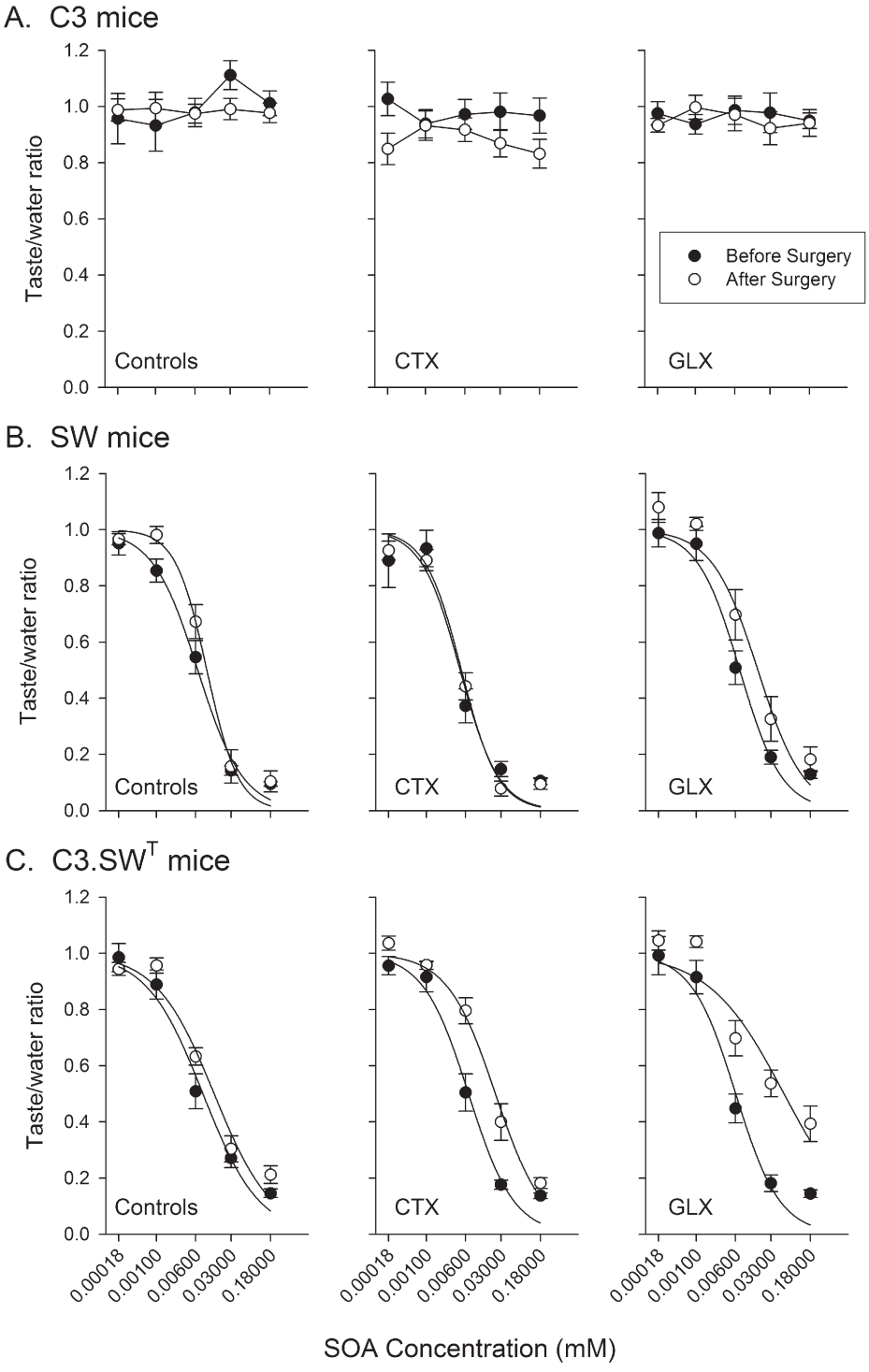

Figure 4 Mean (±SE) standardized licks to SOA before (filled circles) and after (open circles) sham surgery (CON), chorda tympani transection (CTX) and glossopharyngeal transection (GLX). **(A)** C3 mice; **(B)** SW mice; **(C)** C3.SW^T mice. Sigmoidal curves were fit to the mean data (see text for details).

(The C3.SW^T shift represents an underestimate, because one of the 10 mice in this group became so insensitive to SOA after surgery that a sigmoidal curve could not be fit to the data and so the average shift is computed across nine mice.) The C3.SW^T mice averaged a 0.57 log unit shift after CTX.

A more conservative assessment of the effect of nerve transection is to compare the effect of surgery on the curve shift:

$$\text{shift} = \log(c)\text{post} - \log(c)\text{pre} \quad (2)$$

where c is the half maximum parameter from equation (1) fit to each animal's presurgical (pre) and postsurgical (post) SOA data. Separate ANOVAs were then conducted on shift for each strain with surgical group as the only factor. This more conservative analysis failed to find reliable shifts in the SW strain [$F(2, 23) = 2.46$, $P > 0.10$, n.s.], but did confirm a main effect of surgical group in the C3.SW^T strain [$F(2,25) = 7.58$, $P < 0.005$]. *Post hoc* Tukey-HSD tests indicated that the GLX group differed from CON ($P < 0.005$).

The significant effects reported for the main analysis were also statistically significant when only cases with verified histology were analyzed.

The effect of nerve transection on quinine sensitivity

Nerve transection did not reduce quinine avoidance in C3 mice. Surprisingly, C3 mice actually were more sensitive to quinine after GLX [test, $F(1,12) = 12.1$, $P < 0.005$], although the magnitude of this effect was small (Figure 5A). The effect of GLX on the concentration–response functions was also evidenced by a significant change in the logarithm-transformed c-parameter from the individual curve fits [$T(12) = 4.2$, $P < 0.005$]. In addition, CON C3 mice showed a significant test × concentration interaction [$F(4,40) = 3.7$, $P < 0.05$], with again evidence of increased sensitivity with time.

Nerve transection did reduce avoidance of quinine in the other strains (Figure 5B,C). CTX reduced avoidance in both SW mice [test, $F(1,7) = 50.0$, $P < 0.005$] and C3.SW^T mice [test, $F(1,6) = 7.7$, $P < 0.05$]; both findings were reinforced from the analysis on the individual curve fits [SW, $T(7) = 4.5$, $P < 0.005$, mean shift = 0.20 log units; C3.SW^T, $T(6) = 4.0$, $P < 0.01$, mean shift = 0.27 log units]. Finally, GLX caused a significant decrease in sensitivity in the SW mice in one analysis [test × concentration, $F(4,28) = 5.2$, $P < 0.01$] but not in the analysis of individual curve fits [$T(7) = 1.49$, $P > 0.18$, n.s.].

An ANOVA for each strain comparing the curve shift across the surgery groups—see equation (2)—found no main effect of surgery in either the SW or C3.SW^T strain. This analysis is the most conservative estimate of the effect of surgery, because it takes into account any reduced avoidance seen in control mice. The significant effects reported above must therefore be treated with caution.

The significant and nonsignificant results reported above for the main analysis remained so when only histologically verified cases were included, with one exception: the small increase in avoidance of quinine seen in C3 mice no longer reached the statistical rejection criterion.

Discussion

Taste sensitivity to SOA and quinine

The C3 SOA demitaster mice did not avoid any concentration of SOA, whereas SW and C3.SW^T mice avoided 0.006–0.18 mM SOA. Notably, SW and C3.SW^T mice appear to possess identical levels of avoidance of these concentrations, indicating that variation at the *Soa* locus accounts for a robust difference in sensitivity between the progenitor strains. Neurologically intact mice, therefore, show similar levels of SOA avoidance regardless if they have one or two copies of the taster allele (the C3.SW^T mice are heterozygotes at the *Soa* locus). This result agrees with our previous findings using either brief-access or two-bottle tests (Boughter and Whitney, 1998; Boughter *et al.*, 2002). For quinine, three distinct phenotypes were found: SW mice displayed significantly greater avoidance than C3 mice, whereas C3.SW^T mice were intermediate. Thus, variation in the chromosomal region containing *Soa* partially conferred increased taste sensitivity to quinine. No comparable brief-access quinine data have been published; however, similar partial effects were seen using these same strains in two-bottle tests with quinine and 6-*n*-propylthiouracil (Boughter and Whitney, 1998).

The *Soa* locus has been mapped to a ~1 cM interval on mouse chromosome 6 which also contains *Prp*, a locus containing two genes encoding proline-rich salivary proteins (Bachmanov *et al.*, 2001). *Prp* is in turn tightly linked to a cluster of 26 *Tas2r* bitter taste receptor genes (Adler *et al.*, 2000; Shi *et al.*, 2003). Transgenic overexpression of both *Prp* genes in an SOA nontaster strain does not rescue sensitivity to SOA (Harder *et al.*, 2000) and so it is likely that *Soa* corresponds to one or more of these *Tas2r* genes. However, no specific candidate gene has been yet identified that underlies SOA sensitivity.

A locus for quinine aversion as measured by two-bottle tests, termed *Qui*, is also tightly linked to *Prp* and therefore may also represent a *Tas2r* gene (Lush, 1984; Harder and Whitney, 1998; Blizard *et al.*, 1999). Because *Soa* and *Qui* map to the same location, it is highly likely that C3.SW^T mice possess the taster alleles at both loci. There are at least two possible explanations for the fact that the C3.SW^T mice possess an intermediate phenotype to quinine sensitivity in the current study. First, the dominance of the quinine taster allele may be incomplete and possession of only one taster allele may produce the intermediate phenotype. Previously published F_1 heterozygote data for quinine intake are equivocal; dominance effects vary and apparently depend on the inbred strains that are used as parents (Lush, 1984; Boughter *et al.*, 1992; Bachmanov *et al.*, 1996; Blizard *et al.*, 1999). However, F_1 heterozygotes produced from a C3 × SW or 129 × SW cross (129/Sv; quinine non-tasters) were similar in terms of quinine aversion to the SW mice, indicative of complete dominance (Lush, 1984; Boughter *et al.*, 1992). Comparable F_1 data using brief-access tests have not been published, but recently we have shown that F_1 mice from a B6–D2 (C57BL/6J, quinine tasters and DBA/2J, quinine non-tasters) cross are identical to B6 mice in level of aversion in brief-access tests, indicating complete dominance of the taster allele or alleles (data presented at the Association

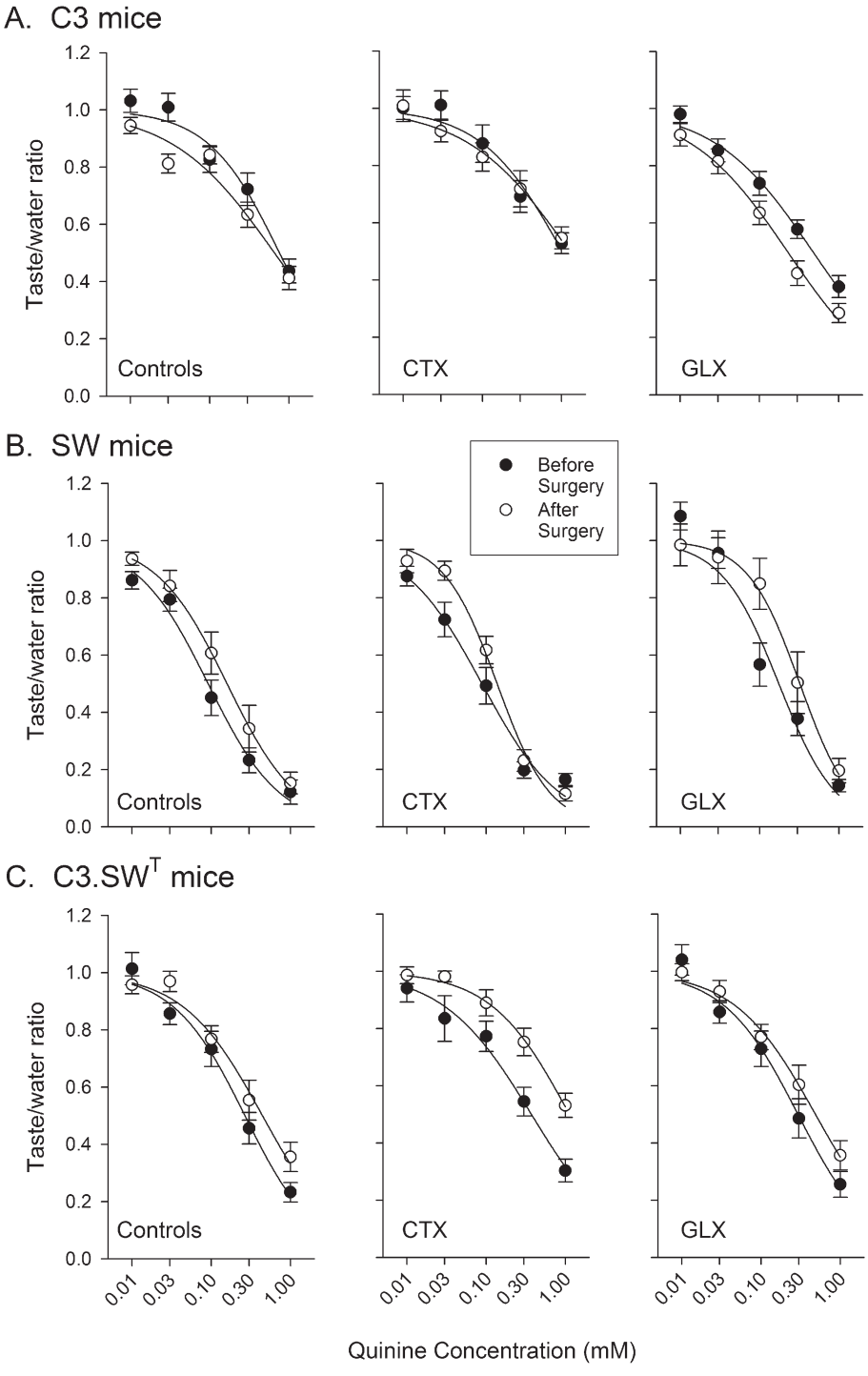

Figure 5 Mean (±SE) standardized licks to quinine before (filled circles) and after (open circles) sham surgery (CON), chorda tympani transection (CTX) and glossopharyngeal transection (GLX). **(A)** C3 mice; **(B)** SW mice; **(C)** C3.SW^T mice. Sigmoidal curves were fit to the mean data (see text for details).

for Chemoreception Sciences Annual Meeting, April 2004, Sarasota, FL).

The second explanation lies in the complex genetic basis of quinine sensitivity: segregation analyses for two-bottle quinine intake were consistent with a polygenic mode of inheritance in two unrelated studies (Boughter *et al.*, 1992; Bachmanov *et al.*, 1996). More recently, Harder and Whitney (1998) measured two-bottle quinine avoidance in BXH/Ty recombinant inbred mice. Intermediate strain values indicated polygenic determination and linkage analysis indicated as many as five and possibly more loci with quantitative effects on quinine aversion. The intake

studies previously done with C3.SW^T mice indicate that a locus on distal mouse chromosome 6 has a relatively large yet quantitative effect on quinine avoidance (Boughter and Whitney, 1998). Thus, the congenic tasters are SW-like at one or possibly two of the loci that contribute to quinine avoidance (i.e. *Soa* and *Qui*), but would be C3-like at other loci. The intermediate phenotype observed would be consistent with polygenic determination of quinine avoidance.

Strain differences in nerve cut effects

Bilateral nerve transection affected sensitivity to SOA in SW and C3.SW^T, but not C3, mice. Interestingly, the magnitude of this effect was generally stronger for C3.SW^T than for SW mice. This finding is noteworthy given that neurologically intact C3.SW^T mice behave exactly like the SW parental strain in both brief-access tests and two-bottle preference tests (Boughter and Whitney, 1995, 1998; Boughter *et al.*, 2002). Nerve transection appears to be unmasking a potentially interesting difference between the congenics and the SW parental strain. Although the *Soa* taster allele appears to show complete dominance in neurologically intact animals, it is possible that, when the system is compromised, phenotypic differences between homozygotes and heterozygotes emerge. These differences might occur particularly if there is a nonlinear relationship between number of taste receptors and taste sensitivity. For example, in the rat, transection of either the GL or the CT has no effect on quinine sensitivity, but combined transection does (St. John *et al.*, 1994). Likewise, removal of taste buds of either the anterior tongue or the hard palate has marginal effects on sucrose sensitivity, whereas the removal of both sets of taste buds has a large effect (Spector *et al.*, 1993). In both cases, sensitivity is not linearly related to behavioral competence—the system survives a small insult but is compromised after a large insult. If C3.SW^T mice transcribe both taste and nontaster alleles in a co-dominant fashion and assuming these alleles represent taste receptors that are polymorphic between SW and C3 mice (e.g. Chandrashekar *et al.*, 2000; Nelson *et al.*, 2003), these heterozygous mice would express receptors that bind SOA at high affinity and other receptors that do not. Like rats with a partial insult to the gustatory system, these C3.SW^T mice may not possess as many functional receptors as the SW mice, but nonetheless show normal behavioral performance. An additional insult to the system, by CTX or GLX (analogous to double denervation in rats), is enough to manifest behavioral differences. Comparison of nerve-transected congenic tasters that are homozygous at the *Soa* locus would provide a test of this interpretation of the data.

A second possibility lies in the 99% C3-like genetic background of the C3.SW^T mice. When expression of the SOA mechanism is compromised, either by removing the anterior or posterior taste bud field, the mice tended to become more C3-like. This effect appears especially robust with GLX, perhaps reflecting the larger number of taste buds innervated by the GL. Virtually any behavioral assay is going to reflect the contribution of many genes and experiences; although the strain differences for SOA are stark in our test, it is important to consider all of the factors that might affect the mouse's behavior. Behavior in this task certainly depends on taste sensitivity, but also depends on hydrational state, attention, olfaction, general activity levels, etc.; performance after surgery additionally depends upon memory of the task, plasticity processes and recovery from the stress of the surgical procedure. It is possible that small differences between C3.SW^T and SW mice in the nerve transection groups reflect the influence of the C3 background on these more difficult to quantify factors.

The effect of nerve transection on quinine responsiveness is somewhat more difficult to assess, because the reliability of the effects of nerve transection on responsiveness varied with the statistical approach employed. In the most conservative analysis, neither nerve cut affected quinine sensitivity. This result is consistent with work on the rat (see below). By a more liberal analysis, C3 mice actually seemed to avoid quinine more after surgery, although the magnitude of this effect was not large. SW and C3.SW^T mice showed decreases in quinine sensitivity, particularly after CTX. By any analysis, the effect of single, bilateral gustatory nerve transection on appetitive responses to quinine were modest, at best, suggesting that, as with rats, hedonically guided ingestive responses in mice do not depend critically on any one taste bud population.

Although some of the nerve cut effects on behavioral responses to tastants were subtle, there was a pronounced and unexpected strain difference in the effect of CTX on the integrity of fungiform papillae. Fungiform papillae are mushroom-shaped protrusions on the anterior tongue that, in rodents, usually house a single taste bud. The taste buds are dependent on an intact CT, but fungiform papillae appear to only partly depend on gustatory innervation. In rats, CTX is associated with a loss of ~25% of fungiform papillae (St. John *et al.*, 1995), in part because some papillae lose their classic morphology and develop filiform spines typical of nongustatory regions of the anterior tongue (Oakley *et al.*, 1990, 1993; St. John *et al.*, 1995). The integrity of fungiform papillae shows a greater dependence on an intact CT in SW mice relative to C3 mice or rats (Table 2). Although these strains have equivalent numbers of fungiform papillae in unmanipulated animals, SW mice had nearly a 60% reduction in papilla number after CTX, whereas C3 and C3.SW^T mice lost 35 and 25%, respectively. Unsurprisingly, the congenic mice were identical to the C3 parental strain on this measure, which is to be expected since the only genetic difference between the congenics and the C3 is in a region of the genome thought to code for T2R receptors. (Although there are other non-T2R coding regions linked to this part of the genome, it would represent a notable coincidence for the congenic mice to be similar to the SW mice on this measure, which does not seem logically

to depend on the presence of particular variants of T2Rs.) For whatever reason, fungiform papillae are more dependent on the CT nerve in SW mice than C3 mice. This effect could be due to a direct trophic function of the nerve for papillae, or could be due to trophic functions of the sublingual and submaxillary salivary glands, which are partially innervated by the CT.

The peripheral organization of bitter taste

The peripheral organization of bitter taste has had a confused history. On one hand, taste researchers have long battled the misleading 'tongue map' often printed in textbooks that erroneously implies that bitter taste is the exclusive purview of posterior tongue receptors (Bartoshuk and Beauchamp, 1994; Lindemann, 2001). Psychophysical evidence to the contrary has existed for decades (Collings, 1974). On the other hand, investigations into taste transduction mechanisms for compounds humans describe as bitter virtually always are performed on posterior lingual tissue, in part because of the high density of taste receptor cells there, but also because of the perceived predominance of bitter taste transduction machinery (Wong *et al.*, 1996; Boughter *et al.*, 1997; Adler *et al.*, 2000) and electrophysiological responsiveness (see Smith and Frank, 1993) of rodent posterior tongue taste buds. The weak response to bitter compounds of nerves subserving the anterior taste buds (Frank *et al.*, 1983; Smith and Frank, 1993; Sollars and Hill, 1998) has reinforced the idea of a tongue map of sorts in rodents; that is, that the posterior tongue predominates in analyzing aversive tastes whereas the anterior oral cavity predominates in analyzing preferred substances.

Despite these logically sound predictions, some behavioral results have supported this view, whereas others have not. In general, transection of the GL has no effects on appetitive tasks (though see Ninomiya *et al.*, 1994; Spector and St. John, 1998; Markison *et al.*, 1999), but has reliable affects on reflexive oromotor behaviors such as gaping (Travers *et al.*, 1987; Grill *et al.*, 1992; King *et al.*, 2000). Transection of the GL does not alter quinine responsivity in two-bottle preference tests (Akaike *et al.*, 1965; Grill *et al.*, 1992), brief access lick rates (Yamamoto and Asai, 1986; St. John *et al.*, 1994), detection of low quinine concentrations (St. John and Spector, 1996), or discrimination of quinine from other aversive substances (St. John and Spector, 1998). These behavioral nerve transection studies were all conducted in rats. Given the potential for control of genetic contributions to taste in the mouse model, we were eager to assess regional sensitivity predictions (based on electrophysiology, immunocytochemistry and *in situ* hybridization) in mice as well. Perfectly consistent with the large body of rat literature, GLX had, at best, only minimal effects on responsiveness to two aversive compounds, SOA and quinine.

A reappraisal of the electrophysiological data in mice presents a more complex picture than is sometimes painted (perhaps even more complex than is the case in rats). First, many studies report only integrated responses of the whole nerve, which can misrepresent the contribution of smaller fibers or fibers distal to the electrode. Secondly, comparisons of the magnitude of an integrated response between different nerves within the same animal or the same nerve between different animals depend crucially on surgical and recording conditions requiring some form of standardization; if the standard stimulus itself has different potencies for the CT and GL then cross-nerve comparisons for particular stimuli can be misinterpreted. Third, posterior receptors, buried in folds in the tongue, may be more poorly stimulated than anterior receptors in anesthetized animals. Finally, though, it is not clear that the CT is uniformly less responsive to 'bitter' compounds than the GL. Thus, where quinine seems to be a poor stimulus for the CT in some studies of mouse gustatory nerves (Shingai and Beidler, 1985), quinine may be nearly as good a stimulus for the CT as the GL in other studies (Inoue *et al.*, 2001; Danilova and Hellekant, 2003). The findings of Inoue *et al.* (2001) are particularly important with regard to the current work, because these investigators tested the response of the GL and CT to SOA and quinine in SW, B6 (C57BL/6ByJ) and SW.B6 congenic mice (where B6 mice are similar to C3 in SOA sensitivity). Although the GL responded better to SOA and quinine than the CT at higher concentrations, both nerves responded well to these compounds at intermediate concentrations and the authors concluded that both nerves contributed to the strain differences in bitter sensitivity of SW and B6 mice.

The idea that elimination of the posterior receptors should reduce (or eliminate) appetitive responses to quinine and SOA is also suggested by the distribution of T2R receptors (Adler *et al.*, 2000). These receptors, argued to collectively account for all sensitivity to compounds considered bitter (Zhang *et al.*, 2003), co-localize on the same taste receptor cells and are not expressed highly in anterior lingual tissue. However, these receptors are expressed in the palate (innervated by the greater superficial petrosal nerve, unmanipulated in our study), which could explain the relatively small effects of GLX on responses to SOA and quinine. More intriguingly, we found that GLX had a larger effect on behavioral responses to SOA than quinine. If T2Rs are always expressed equally on the same cells, one would not expect differential effects of GLX on the two stimuli. However, this result is consistent with the notion that some stimuli avail themselves of T2R-independent transduction mechanisms (Spielman *et al.*, 1992; Glendinning *et al.*, 2000; Peri *et al.*, 2000; Zhao *et al.*, 2002; Caicedo *et al.*, 2003).

Future research may help to eliminate the perceived disparity between behavioral, electrophysiological and anatomical results. For example, given the caveats associated with comparing electrophysiological results between nerves and given the recognition that quinine and other aversive compounds do in fact stimulate the CT (and probably the greater superficial petrosal nerve as well), the ability of the system to survive insult to the GL appears less contro-

versial. Additionally, given the lack of detailed regional distribution studies of T2Rs in the mouse, it is plausible that we will obtain a more coherent picture of the peripheral organization of bitter taste as more data accrue.

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Both authors contributed equally to this project.

References

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J. and **Zuker, C.S.** (2000) *A novel family of mammalian taste receptors*. Cell, 100, 693–702.

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

Bachmanov, A.A., Reed, D.R., Tordoff, M.G., Price, R.A. and **Beauchamp, G.K.** (1996) *Intake of ethanol, sodium chloride, sucrose, citric acid and quinine hydrochloride solutions by mice: a genetic analysis*. Behav. Genet., 26, 563–573.

Bachmanov, A.A., Li, X., Li, S., Neira, M., Beauchamp, G.K. and **Azen, E.A.** (2001) *High-resolution genetic mapping of the sucrose octaacetate taste aversion (Soa) locus on mouse chromosome 6*. Mamm. Genome, 12, 695–699.

Bartoshuk, L.M. and **Beauchamp, G.K.** (1994) *Chemical senses*. Annu. Rev. Psychol., 45, 419–449.

Blizard, D.A., Kotlus, B. and **Frank, M.E.** (1999) *Quantitative trait loci associated with short-term intake of sucrose, saccharin and quinine solutions in laboratory mice*. Chem. Senses, 24, 373–385.

Boudreau, J.C., Sivakumar, L., Do, L.T., White, T.D., Oravec, J. and **Hoang, N.K.** (1985) *Neurophysiology of geniculate ganglion (facial nerve) taste systems: species comparisons*. Chem. Senses, 10, 89–127.

Boudreau, J.C., Do, L.T., Sivakumar, L., Oravec, J. and **Rodriquez, C.** (1987) *Taste systems of the petrosal ganglion of the rat glossopharyngeal nerve*. Chem. Senses, 12, 437–458.

Boughter, J.D. Jr and **Whitney, G.** (1995) *C3.SW-Soaa heterozygous congenic taster mice*. Behav. Genet., 25, 233–237.

Boughter, J.D. Jr and **Whitney, G.** (1998) *Behavioral specificity of the bitter taste gene Soa*. Physiol. Behav., 63, 101–108.

Boughter, J.D., Harder, D.B., Capeless, C.G. and **Whitney, G.** (1992) *Polygenic determination of quinine aversion among mice*. Chem. Senses, 17, 427–434.

Boughter, J.D. Jr, Pumplin, D.W., Yu, C., Christy, R.C. and **Smith, D.V.** (1997) *Differential expression of alpha-gustducin in taste bud populations of the rat and hamster*. J. Neurosci., 17, 2852–2858.

Boughter, J.D. Jr, St John, S.J., Noel, D.T., Ndubuizu, O. and **Smith, D.V.** (2002) *A brief-access test for bitter taste in mice*. Chem. Senses, 27, 133–142.

Caicedo, A., Pereira, E., Margolskee, R.F. and **Roper, S.D.** (2003) *Role of the G-protein subunit alpha-gustducin in taste cell responses to bitter stimuli*. J. Neurosci., 23, 9947–9952.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S. and **Ryba, N.J.** (2000) *T2Rs function as bitter taste receptors*. Cell, 100, 703–711.

Collings, V. (1974) *Human taste response as a function of locus of stimulation on the tongue and soft palate*. Percept. Psychophys., 16, 169–174.

Danilova, V. and **Hellekant, G.** (2003) *Comparison of the responses of the chorda tympani and g*lossopharyngeal nerves to taste stimuli in C57BL/6J mice. BMC Neurosci., 4, 5.

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

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

Ganchrow, J.R. and **Ganchrow, D.** (1989) *Long-term effects of gustatory neurectomy on fungiform papillae in the young rat*. Anat. Rec., 225, 224–231.

Glendinning, J.I., Chaudhari, N. and **Kinnamon, S.C.** (2000) *Taste transduction and molecular biology.* In Finger, T.E., Silver, W.L. and Restrepo, D. (eds), The Neurobiology of Taste and Smell. New York: Wiley-Liss, pp. 315–351.

Grill, H.J. and **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 Res., 573, 105–113.

Grill, H.J., Schwartz, G.J. and **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 Res., 573, 95–104.

Hallagan, L., Saperstein, S. and **St John, S.J.** (2003) *Cetylpyridinium chloride's aversive taste to rats*. Chem. Senses., 28, A91.

Harder, D.B. and **Whitney, G.** (1998) *A common polygenic basis for quinine and PROP avoidance in mice*. Chem. Senses, 23, 327–332.

Harder, D.B., Azen, E.A. and **Whitney, G.** (2000) *Sucrose octaacetate avoidance in nontaster mice is not enhanced by two type-A Prp transgenes from taster mice*. Chem. Senses, 25, 39–45.

Inoue, M., Li, X., McCaughey, S.A., Beauchamp, G.K. and **Bachmanov, A.A.** (2001) *Soa genotype selectively affects mouse gustatory neural responses to sucrose octaacetate*. Physiol. Genomics, 5, 181–186.

Jacquin, M.F. (1983) *Gustation and ingestive behavior in the rat*. Behav. Neurosci., 97, 98–109.

King, C.T., Garcea, M. and **Spector, A.C.** (2000) *Glossopharyngeal nerve regeneration is essential for the complete recovery of quinine-stimulated oromotor rejection behaviors and central patterns of neuronal activity in the nucleus of the solitary tract in the rat*. J. Neurosci., 20, 8426–8434.

Lindemann, B. (2001) *Receptors and transduction in taste*. Nature, 413, 219–225.

Lush, I.E. (1984) *The genetics of tasting in mice. III. Quinine*. Genet. Res., 44, 151–160.

Markison, S., St. John, S.J. and **Spector, A.C.** (1999) *Glossopharyngeal nerve transection reduces quinine avoidance in rats not given presurgical stimulus exposure*. Physiol. Behav., 65, 773–778.

Matsunami, H., Montmayeur, J.P. and **Buck, L.B.** (2000) *A family of candidate taste receptors in human and mouse*. Nature, 404, 601–604.

Ming, D., Ninomiya, Y. and **Margolskee, R.F.** (1999) *Blocking taste receptor activation of gustducin inhibits gustatory responses to bitter compounds*. Proc. Natl Acad. Sci. USA, 96, 9903–9908.

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

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J. and **Zuker, C.S.** (2001) *Mammalian sweet taste receptors*. Cell, 106, 381–390.

Nelson, T.M., Munger, S.D. and **Boughter, J.D. Jr** (2003) *Taste sensitivities to PROP and PTC vary independently in mice*. Chem. Senses, 28, 695–704.

Ninomiya, Y., Tonosaki, K. and **Funakoshi, M.** (1982) *Gustatory neural response in the mouse*. Brain Res., 244, 370–373.

Ninomiya, Y., Mizukoshi, T., Higashi, T., Katsukawa, H. and **Funakoshi, M.** (1984) *Gustatory neural responses in three different strains of mice*. Brain Res., 302, 305–314.

Ninomiya, Y., Kajiura, H., Naito, Y., Mochizuki, K., Katsukawa, H. and **Torii, K.** (1994) *Glossopharyngeal denervation alters responses to nutrients and toxic substances*. Physiol. Behav., 56, 1179–1184.

Oakley, **B.** (1967) *Altered temperature and taste responses from cross-regenerated sensory nerves in the rat's tongue*. J. Physiol., 188, 353–371.

Oakley, B., Wu, L.H., Lawton, A. and **deSibour, C.** (1990) *Neural control of ectopic filiform spines in adult tongue*. Neuroscience, 36, 831–838.

Oakley, B., Lawton, A., Riddle, D.R. and **Wu, L.H.** (1993) *Morphometric and immunocytochemical assessment of fungiform taste buds after interruption of the chorda-lingual nerve*. Microsc. Res. Tech., 26, 187–195.

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

Parks, **J.D.** and **Whitehead, M.C.** (1998) *Scanning electron microscopy of denervated taste buds in hamster: morphology of fungiform taste pores*. Anat. Rec., 251, 230–239.

Peri, I., Mamrud-Brains, H., Rodin, S., Krizhanovsky, V., Shai, Y., Nir, S. and **Naim, M.** (2000) *Rapid entry of bitter and sweet tastants into liposomes and taste cells: implications for signal transduction*. Am. J. Physiol. Cell Physiol., 278, C17–C25.

Pfaffmann, **C.** (1952) *Taste preference and aversion following lingual denervation*. J. Comp. Physiol. Psychol., 45, 393–400.

Pfaffmann, **C.** (1955) *Gustatory nerve impulses in rat, cat and rabbit*. J. Neurophysiol., 18, 429–440.

Rhinehart-Doty, J.A., Schumm, J., Smith, J.C. and **Smith, G.P.** (1994) *A non-taste cue of sucrose in short-term taste tests in rats*. Chem. Senses, 19, 425–431.

Shi, P., Zhang, J., Yang, H. and **Zhang, Y.P.** (2003) *Adaptive diversification of bitter taste receptor genes in mammalian evolution*. Mol. Biol. Evol., 20, 805–814.

Shingai, **T.** and **Beidler, L.M.** (1985) *Response characteristics of three taste nerves in mice*. Brain Res., 335, 245–249.

Smith, **D.V.** and **Frank, M.E.** (1993) *Sensory coding by peripheral taste fibers.* In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL, pp. 295–336.

Smith, **J.C.** (2001) *The history of the 'Davis Rig'*. Appetite, 36, 93–98.

Sollars, **S.I.** and **Hill, D.L.** (1998) *Taste responses in the greater superficial petrosal nerve: substantial sodium salt and amiloride sensitivities demonstrated in two rat strains*. Behav. Neurosci., 112, 991–1000.

Spector, **A.C.** and **St. John, S.J.** (1998) *Role of taste in the microstructure of quinine ingestion by rats*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 274, R1687–R703.

Spector, A.C., Travers, S.P. and **Norgren, R.** (1993) *Taste receptors on the anterior tongue and nasoincisor ducts of rats contribute synergistically to behavioral responses to sucrose*. Behav. Neurosci., 107, 694–702.

Spielman, A.I., Huque, T., Whitney, G. and **Brand, J.G.** (1992) *The diversity of bitter taste signal transduction mechanisms.* In Corey, D. and Roper, S.D. (eds), Sensory Transduction. Rockefeller University Press, New York, pp. 307–324.

St. John, **S.J.** and **Spector, A.C.** (1996) *Combined glossopharyngeal and chorda tympani nerve transection elevates quinine detection thresholds in rats (*Rattus norvegicus*)*. Behav. Neurosci., 110, 1456–1468.

St. John, **S.J.** and **Spector, A.C.** (1998) *Behavioral discrimination between quinine and KCl is dependent on input from the seventh cranial nerve: implications for the functional roles of the gustatory nerves in rats*. J. Neurosci., 18, 4353–4362.

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

St. John, S.J., Markison, S. and **Spector, A.C.** (1995) *Salt discriminability is related to number of regenerated taste buds after chorda tympani nerve section in rats*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 269, R141–R153.

Travers, J.B., Grill, H.J. and **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*. Behav. Brain Res., 25, 233–246.

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

Whitehead, M.C., Frank, M.E., Hettinger, T.P., Hou, L.T. and **Nah, H.D.** (1987) *Persistence of taste buds in denervated fungiform papillae*. Brain Res., 405, 192–195.

Whitney, **G.** and **Harder, D.B.** (1994) *Genetics of bitter perception in mice*. Physiol. Behav., 56, 1141–1147.

Wong, G.T., Gannon, K.S. and **Margolskee, R.F.** (1996) *Transduction of bitter and sweet taste by gustducin*. Nature, 381, 796–800.

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

Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S. and **Ryba, N.J.** (2003) *Coding of sweet, bitter and umami tastes. Different receptor cells sharing similar signaling pathways*. Cell, 112, 293–301.

Zhao, F.L., Lu, S.G. and **Herness, S.** (2002) *Dual actions of caffeine on voltage-dependent currents and intracellular calcium in taste receptor cells*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 283, R115–R129.

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