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Effects of Chorda Tympani Nerve Anesthesia on Taste Responses in the NST

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Abstract

Human clinical and psychophysical observations suggest that the taste system is able to compensate for losses in peripheral nerve input, since patients do not commonly report decrements in whole mouth taste following chorda tympani nerve damage or anesthesia. Indeed, neurophysiological data from the rat nucleus of the solitary tract (NST) suggests that a release of inhibition (disinhibition) may occur centrally following chorda tympani nerve anesthesia. Our purpose was to study this possibility further. We recorded from 59 multi- and single-unit taste-responsive sites in the rat NST before, during and after recovery from chorda tympani nerve anesthesia. During anesthesia, average anterior tongue responses were eliminated but no compensatory increases in palatal or posterior tongue responses were observed. However, six individual sites displayed increased taste responsiveness during anesthesia. The average increase was 32.9%. Therefore, disinhibition of taste responses was observed, but infrequently and to a small degree in the NST. At a subset of sites, chorda tympani-mediated responses decreased while greater superficial petrosal-mediated responses remained the same during anesthesia. Since this effect was accompanied by a decrease in spontaneous activity, we propose that taste compensation may result in part by a change in signal-to-noise ratio at a subset of sites.

Introduction

Patients who suffer from chorda/lingual nerve damage due to trauma, surgery, infection or pathosis do not typically report deficits in taste (Rice, 1963; Bull, 1965; reviewed in Miller and Bartoshuk, 1991). Although testing discrete taste bud subpopulations reveals an absence of taste sensation on the denervated areas, whole mouth taste perception appears to be normal. In fact, when tested using the 'sip and spit' technique, intensity ratings for a variety of tastants are modified only slightly following anesthetization of the chorda/lingual or chorda tympani nerve (CTN) (Miller and Bartoshuk, 1991; Catalanotto *et al.*, 1993; Lehman *et al.*, 1995). This phenomenon is referred to as 'taste constancy' (Lehman *et al.*, 1995) and, based on early neurophysiological observations (Halpern and Nelson, 1965), has been hypothesized to result from the removal of putative inhibitory CTN influences on cells that receive excitatory inputs from other gustatory nerves within the nucleus of the solitary tract (NST) (Miller and Bartoshuk, 1991; Catalanotto *et al.*, 1993; Lehman *et al.*, 1995). Additional psychophysical evidence for 'release of inhibition' was obtained in a recent experiment in which CTN anesthesia produced increases in the perceived intensity of quinine applied to the circumvallate papillae (Lehman *et al.*, 1995).

The consequences of CTN anesthesia in humans appear to fit nicely with neurophysiological findings in rats reported more than 30 years ago (Halpern and Nelson, 1965). In a classic study of the first-order gustatory relay, the NST, Halpern and Nelson (1965) reported that posterior tongue taste responses increased after CTN anesthesia. These observations would suggest that plasticity capable of compensating for partial taste loss exists in the initial stages of central processing. However, because the main purpose of their study was to investigate gustatory topography and chemosensitivity, many questions regarding anesthetic effects persisted. Importantly, neither the frequency nor magnitude of the residual response increases was clear because CTN anesthetic effects were tested at only a few recording sites. In addition, the identity of the taste receptors giving rise to the enhanced responses was unknown. The authors used an anterior tongue chamber to specifically stimulate only taste buds on the anterior tongue. However, taste buds outside the anterior tongue chamber were non-specifically stimulated. Because the distribution of palatal taste buds was not considered at that time, it was presumed that the taste buds which were stimulated outside the chamber were foliate and circumvallate receptors on the posterior tongue. However, responses could also have arisen from palatal taste buds, which comprise 17% of oral taste buds in the rat (reviewed in Travers and Nicklas, 1990). Thus, during CTN anesthesia taste responses arising from the palate may increase in addition to those from the posterior tongue.

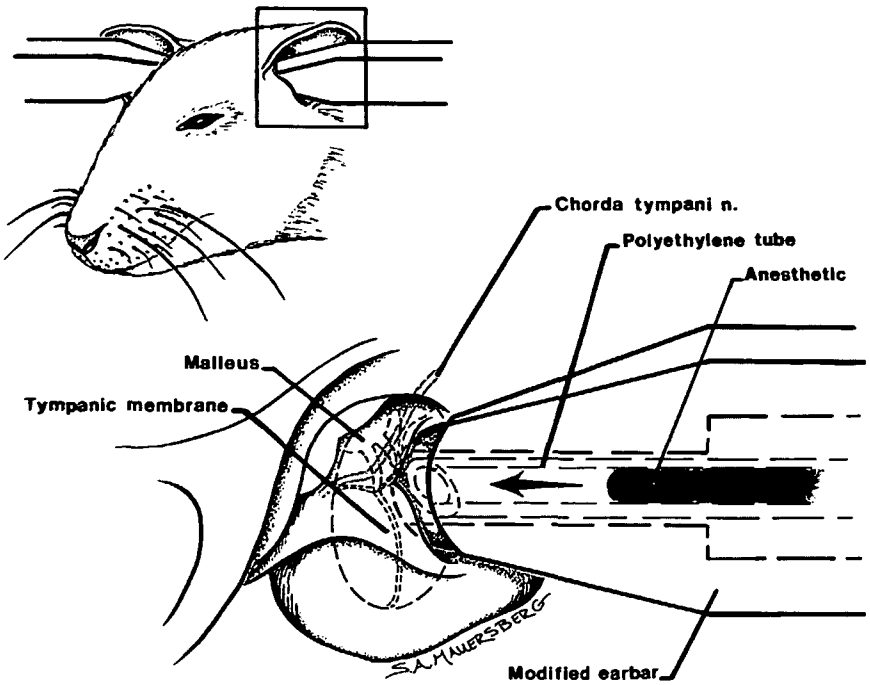


Figure 1 Modified earbars were placed in the external auditory meatus which allowed for convenient and reliable anesthesia of the chorda tympani nerve. A PE-50 cannula was used to administer a small amount (0.02 ml) of lidocaine to the external auditory meatus. The close proximity of the cannula orifice and tympanic membrane allow for rapid diffusion of anesthetic through the perforation to anesthetize the CTN.

The aim of the present study was to more fully investigate the acute effects of CTN anesthesia on gustatory responses in the rat NST. Specifically, we wanted to determine the frequency of disinhibition, quantify its magnitude and characterize which taste bud subpopulations were involved. On the basis of previous neurophysiological and psychophysical results, we hypothesized that disinhibition would occur frequently, would be robust and would arise from posterior tongue stimulation.

Methods and materials

Subjects and anesthesia

Thirty-seven adult male Sprague–Dawley rats (287–601 g) were used in this study. Animals were anesthetized with ethyl carbamate (urethane, 1 g/kg, i.p.) and sodium pentobarbital (Nembutal, 25 mg/kg, i.p.) to achieve a surgical level of anesthesia. This was characterized by an absence of pedal withdrawal upon pinching and lack of corneal blink reflex, and was maintained throughout the experiment with supplemental doses of Nembutal. Animal procedures were approved by the Ohio State University's Laboratory Animal Care and Use Committee.

Neurophysiologic surgical preparation

Surgical preparatory procedures for acute neurophysiologic recording were similar to those described in previous work from this laboratory (e.g. Travers *et al.*, 1986; Travers and Norgren, 1995). An exception to this was the initial perforation of the tympanic membrane ventral to the malleus using a sharp retraction needle. Great care was taken during this procedure to avoid damaging the chorda tympani nerve, which lies medial to the malleus. The perforation allowed for rapid diffusion of lidocaine and saline to the CTN. Animals were placed on a heating pad to keep them near a constant rectal temperature of 37°C. They were stabilized on a stereotaxic apparatus using a mouthpiece and atraumatic earbars (Kopf instruments, Tujunga, CA) which were modified so that a plastic cannula (PE-50; Becton Dickinson, NJ) could be introduced into the external auditory meatus while the head remained stable in the stereotaxic (see Figure 1). The hollow earbars were a modified version from a schematic drawn by Norgren (personal communication). The animal's head was leveled with respect to lambda and bregma landmarks in the horizontal plane. A head holder device was fastened to one earbar and attached to the skull via small bone fixation screws and secured with methyl methacrylate. The advantage of this head holder is the stabilization of the rat's head during recording, enabling the stimulation of discrete taste bud subpopulations in the oral cavity (described in Travers *et al.*, 1986). A tracheal cannula was placed to allow for unimpeded respiration during fluid delivery. An oral drain tube, used to evacuate excess fluid, was placed exiting the same ventral incision (modified from Halpern and Nelson, 1965). The superior laryngeal nerves were routinely

transected; the hypoglossal nerves were transected some of the time. Sutures were placed at four sites around the oral cavity and through the tongue to allow adequate access for the stimulation of different taste bud subpopulations (Travers *et al.*, 1986; Halsell *et al.*, 1993). A craniotomy was performed posterior to lambda in order to access the brain for microelectrode penetration. Physiologic saline was applied to the exposed area of the cerebellum.

Neurophysiologic recording session

Glass-coated tungsten microelectrodes (0.4–2.4 MΩ) were used to record multi- and single-unit neural activity. Neural activity was amplified, observed on an oscilloscope and recorded on VHS tapes for off-line analysis. Recording sites were marked with electrolytic lesions made with anodal current (3 μA, 3 s, Grass stimulator) at the recording site or at a site that was typically 200–400 μm ventral to it.

Taste stimulation

In the main set of experiments (n = 36) responses to gustatory stimulation of the whole mouth, anterior tongue, nasoincisor ducts and foliate papillae were tested. Occasionally, other taste bud subpopulations (soft palate, sublingual organ, retromolar mucosa) were also stimulated. Testing commenced with stimulating the whole mouth with a mixture of tastants (0.3 M sucrose, 0.3 M NaCl, 0.01 M HCl and 0.003 M quinine-hydrochloride) and then individual taste bud subpopulations were tested. Whole mouth stimulation consisted of sequentially flowing 2 ml of water, 2 ml of taste mixture and then 4 ml of water rinse over the lingual, palatal and buccal mucosa using a syringe. Individual taste bud subpopulations were stimulated in a similar water–stimulus–rinse sequence. Small amounts of water and then mixture were applied to the taste buds of interest with a nylon brush and then the whole mouth was rinsed with water from a syringe (Travers *et al.*, 1986; Travers and Norgren, 1995).

In a separate subset of animals (n = 5), sites responsive to circumvallate gustatory stimulation were identified by placing a modified glass pipette in the trench surrounding this papilla to provide for adequate stimulation of these receptors, which are located in the walls of the trench (Frank, 1991; Halsell and Travers, 1997). Because the pipette assembly made it awkward to stimulate other taste bud groups, only whole mouth and circumvallate responses were routinely tested and recorded in these preparations. However, before formal testing commenced, additional taste bud subpopulations were always screened for a response. Sometimes responses to foliate stimulation were present, and in these cases, foliate responsiveness was also tested.

For each stimulus trial, spontaneous activity was recorded for 5–10 s preceding stimulation, and the water, tastant and rinse applications were of equal duration. Neural activity was allowed to return to baseline before the next stimulation (usually at least 60 s). Mechanoreceptive responses were noted but not systematically tested.

Chorda tympani anesthetization

The CTN was anesthetized by administering ~0.02 ml of 2% lidocaine into the external auditory meatus which would then diffuse across the perforated tympanic membrane and anesthetize the nerve, commonly within 10–20 s. In order to hasten recovery from anesthesia, 1–2 ml of physiologic saline was delivered via the same route after testing. The duration of CTN anesthesia exceeded the duration of the stimulation protocol (~15 min or less) and recovery typically required 5–10 min once the nerve was rinsed with saline. The introduction of lidocaine and physiologic saline was via a polyethylene tube attached to a 1 ml syringe in which the tube was advanced through a hollow earbar until the end of the tube was flush with the blunt end of the earbar (see Figure 1). For each preparation, the anesthesia and recovery procedure was initially tested at a site within the NST responsive to gustatory stimulation of the anterior tongue to establish the parameters (volumes of solutions and time) for reliable anesthetization and recovery. Responses to stimulation of the whole mouth and individual taste bud subpopulations with taste mixture before, during and after recovery from chorda tympani anesthesia were tested at each site. The post-anesthesia testing was important for establishing the variability of the responses in the unanesthetized state. Whenever possible, the entire stimulus protocol was repeated.

Histologic reconstruction of recording sites

After the recording session, animals received a lethal dose of sodium pentobarbital (150 mg/kg). They were then perfused intracardially with physiologic saline (300–400 ml) and fixed with 10% buffered formalin (200–300 ml). The brain was dissected from the cranium and stored in a 10% formalin:20% sucrose mixture for cryoprotection. Brains were sectioned at 52 μm on a freezing microtome and mounted on chrome–alum-coated slides, and alternate sections were stained for Nissl substance (cresylecht violet) or myelin (Weil). Recording sites were reconstructed by tracing brainstem sections through the microscope, which was interfaced with a computer using commercially available hardware and software (Vidlucida, Microbrightfield, Colchester, VT). Electrolytic lesions (~100–150 μm in diameter) were identified on cresylecht violet and Weil- stained sections and traced relative to the NST, solitary tract, vestibular nuclei, spinal trigeminal tract and brainstem outline. The location of the recording sites in the antero-posterior and medio-lateral dimensions were transposed to a schematic outline of the NST in the horizontal plane (adapted from Hamilton and Norgren, 1984).

Quantification of neural activity

Recorded neural activity was analyzed off-line. Single-unit activity was differentiated with a window discriminator using consistency of amplitude and waveform as criteria. Multi-unit activity was differentiated by setting the lower level of the window discriminator just above the background level (see Dickman and Smith, 1989; Halsell and Frank, 1992; Halsell *et al.*, 1993). Because the analyses in this study involved comparing responses at the same recording sites during different anesthetic states, normalization was not necessary for the multi-unit responses. Both single- and multi-unit activity were quantified by converting action potentials to digital pulses and accumulating these in 500 ms bins in peristimulus time histograms.

Net-evoked activity was quantified by using a standard response measure, defined as the number of spikes over a 5 or 10 s period (10 s were used when available) during taste stimulation minus the number of spikes that occurred during the preceding water stimulation. This measure was then converted to spikes/s. An exception to this definition was for circumvallate-elicited responses. These were calculated as the mixture response minus spontaneous, rather than water-evoked, activity due to the frequent occurrence of a large but transient mechanoreceptive and/or thermal response to fluid onset. The initial water flow typically evoked a response but it adapted quickly. If a second water stimulation immediately followed the initial water stimulation, a second response was not evident. Thus, subtracting the water response would have underestimated the gustatory contribution. This situation was unique for circumvallate stimulation through the pipette. Transient responses to fluid stimulation sometimes occurred when stimulating other taste bud groups, but in these cases the water and gustatory stimulations were discontinuous, preventing somatosensory adaptation. The criteria for a suprathreshold taste response were defined as a minimum 1 spike/s change in activity, which also had to be >2.5 times the standard deviation of the spontaneous rate (Travers and Smith, 1984; Travers *et al.*, 1986; Travers and Norgren, 1991, 1995).

In addition to the standard measure just described, we used a second measure of taste-evoked activity that we have termed the *relative taste response*. This is the standard response divided by spontaneous activity. Since CTN anesthesia often caused marked decrements in spontaneous activity, analyzing relative responses had the potential to reveal response changes otherwise unapparent using the standard measure analysis. Although the standard measure also incorporates changes in spontaneous activity, it represents net-evoked spikes, and does not fully reflect certain proportional changes in responsiveness that occurred during anesthetization. For example, at recording sites responsive to both anterior tongue and nasoincisor duct stimulation, the spontaneous rate sometimes decreased to near zero. Using the standard measure, net nasoincisor-evoked activity usually remained unchanged. Viewed from another perspective, however, it could be argued that the nasoincisor response actually increased during anesthesia, since the same number of spikes were evoked relative to a lower baseline. The relative response measure quantifies this putative increase.

Stability of taste responses

It was critical to determine whether changes observed during CTN anesthesia were due to anesthetization and not simply to variation over the course of testing. To this end, we compared responses in the anesthetized state with those both prior to and after recovery from anesthesia. As a result, we excluded sites with responses that we defined as unstable. Whole mouth responses were used to determine stability except at circumvallate-responsive sites, where circumvallate responses were used since they were more reliable. Unstable whole mouth responses were defined as sites where responses occurred before anesthesia but not after recovery, or where the percent change in the unanesthetized state exceeded 50% of the mean unanesthetized response:

$$\%\ \text{change in unanesthetized state} = (\text{Pre} - \text{Post})/((\text{Pre} + \text{Post})/2) \times 100$$

where Pre = response before CTN anesthesia and Post = response after recovery from CTN anesthesia.

Using this criterion, the recording sites retained exhibited a mean change in responsiveness in the unanesthetized state of 18.5 ± 12.4% (SD), with a quarter of the sites varying by 10% or less and over half of the sites (61%) by 20% or less. Figure 2 depicts individual responses before, during and

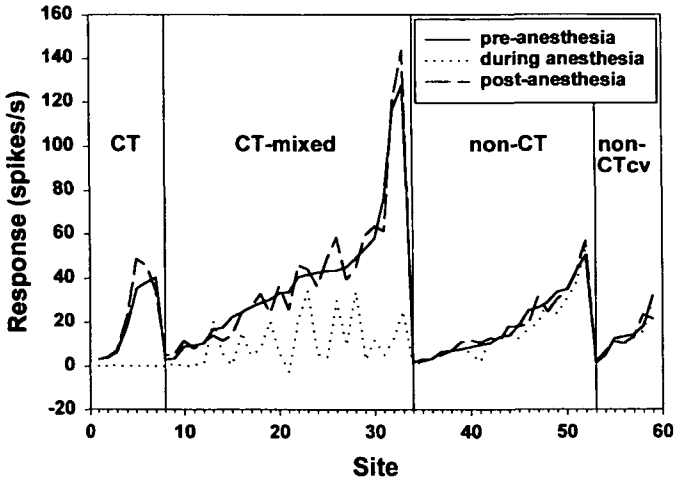


Figure 2 Individual whole mouth taste responses before, during and after recovery from CTN anesthesia by group. The responses (spikes/s) for all 59 sites are depicted. Note the reliability in responses across the unanesthetized state (pre- versus post-anesthesia) and the efficacy of anesthesia apparent as total or partial response decrements for the CT and CT-mixed sites respectively.

after recovery from anesthesia, and suggests a high degree of response stability prior to and after recovery from anesthesia. Across sites, pre- and post-anesthesia responses were very highly correlated ($r = +0.98$, $P < 0.0005$).

Statistical analyses

Sites were categorized into one of three groups based upon their gustatory receptive field response prior to CTN anesthesia since chorda tympani-mediated responses were expected to be eliminated whereas non-chorda tympani-mediated responses were expected to remain the same or increase during CTN anesthesia. Sites which responded only to stimulation of taste bud subpopulations innervated by the CTN (e.g. anterior tongue, sublingual organ, retromolar mucosa) were placed in the CT group. Sites which responded to taste bud subpopulations innervated by the CTN plus another nerve (i.e. glossopharyngeal or greater superficial petrosal) were included in the CT-mixed group. Sites which responded to a taste bud subpopulation innervated solely (nasoincisor duct, circumvallate papilla, soft palate) or principally (foliate papillae) by a nerve other than the CTN were classified as 'non-chorda tympani' (non-CT). A subset of sites ($n = 7$) within the non-CT group which responded to circumvallate stimulation were also referred to as non-CT_{cv}. One potential complication for this scheme is that the foliate papillae also receive a minor innervation from the CTN (Whiteside, 1927; Yamamoto and Kawamura, 1975; Miller *et al.*, 1978). This issue is addressed in Discussion.

Repeated measures ANOVAs were performed to compare the three anesthetic conditions. Separate analyses were done for each taste bud subpopulation and spontaneous activity for each group of recording sites. Multi- and single-unit sites were collapsed for these analyses because nearly identical results were obtained when the analysis was restricted to single units. ANOVAs were followed by post-hoc contrasts comparing pre- and post-anesthesia responses with each other and with the responses in the anesthetized condition. Probability values for contrasts were Bonferonni-adjusted, and significance levels set at $P \leq 0.05$. Unless noted, the P values in the text are the adjusted P values for the contrasts, which assume a significant main effect for anesthetic condition. In a few instances it was of interest to compare the magnitude of responses for different types of recording sites. These comparisons were performed using t-tests and restricted to single-unit sites. Unless stated otherwise, variances are reported as SEMs.

Analysis of individual recording sites

The strength of analyzing the average responses was that enough data were available to use inferential statistical methods. However, a potential limitation was that we might have missed effects that occurred in specific subsets of the sample. For example, if responses at one-third of the recording sites increased while another third decreased by a similar amount during anesthesia, it would have appeared as though no changes had occurred overall, even though they had changed in a significant percentage of the population. Because insufficient data for standard statistics were available for individual sites, an alternative criterion was developed for making reasonable judgements about whether a change in responsiveness occurred during anesthesia. The criterion used the standard deviation of the gustatory responses before and after recovery from CTN anesthesia as a measure of variability in the unanesthetized state. If the response during anesthesia deviated from the mean of the pre- and post-anesthesia responses by more than two standard deviations, the response was considered to have been altered during the anesthetic state. For sites which met this criterion, the change in response was calculated using the following formula:

$$\%\ \text{change during anesthesia} = (\text{Anesth} - (\text{Pre} + \text{Post})/2)/((\text{Pre} + \text{Post})/2) \times 100$$

where Anesth = response during CTN anesthesia, Pre = response before CTN anesthesia and Post = response after recovery from CTN anesthesia.

Results

Anatomical location

Gustatory responses from 59 multi- and single-unit sites were recorded before, during and after recovery from CTN anesthesia. Subsequent to recording, electrolytic lesions were made either at the site of recording or 200–400 μm ventral to it. Based on histologic reconstruction of 41 sites, all appeared to be within the boundaries of the NST (see Figure 3). A topographic organization was observed, with CT and CT-mixed sites predominantly anterior and lateral to non-CT sites. Since all CT and most CT-mixed sites responded mainly to anterior oral cavity stimulation whereas a majority of non-CT sites responded to posterior oral cavity stimulation (see below), this organization is similar to previous descriptions of NST orotopy from this laboratory (Travers *et al.*, 1986; Travers and Norgren, 1995).

Classification of recording sites

Approximately equal numbers of recordings were obtained from multi- ($n = 30$) and single-unit ($n = 29$) sites; however, these sites were unevenly dispersed among groups. Seven sites were classified as CT; each was a single-unit site. Twenty-six sites were placed in the CT-mixed group, including 10 single cells. The remaining 26 sites were non-CT sites; 12 were single units. All of the CT sites responded to gustatory stimulation of the whole mouth and anterior tongue only. The sites categorized as CT-mixed and non-CT were more complex. Most CT-mixed sites ($n = 21$) responded to whole mouth, anterior tongue and nasoincisor duct stimulation. Many non-CT sites responded to

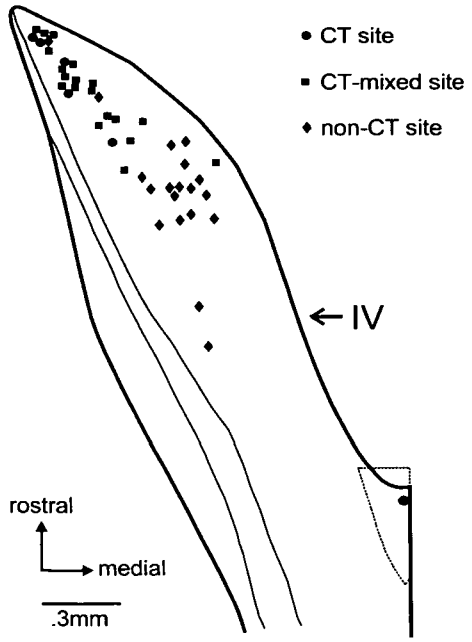


Figure 3 Recording sites were reconstructed on a horizontal schematic of the NST. A total of 41 sites are depicted. Symbols for the sites are based upon the response categories described in the text. Note that an orotopic organization of taste responses was found as described previously by Travers and Norgren (1995).

Table 1 Taste bud subpopulation responses categorized by group

Taste bud subpopulation	CT	CT-mixed	non-CT	Total
WM/AT	7(7)			7(7)
WM/AT/NID		21(8)		21(8)
WM/AT/NID/FOL		2(0)		2(0)
WM/RM/SP		1(1)		1(1)
WM/SLO/NID		1(1)		1(1)
WM/AT/NID/FOL/SP		1(0)		1(0)
WM/FOL			12(7)	12(7)
WM/SP			3(1)	3(1)
WM/NID			2(2)	2(2)
WM			1(0)	1(0)
WM/NID/FOL			1(1)	1(1)
WM/CV			4(0)	4(0)
CV			2(1)	2(1)
WM/FOL/CV			1(0)	1(0)
Total	7(7)	26(10)	26(12)	59(29)

All of the sites are described by taste bud subpopulations and categorized accordingly into CT, CT-mixed and non-CT groups. The first number indicates multi- and single-unit sites combined whereas the number in parentheses indicates single cells only. The following abbreviations are used: WM = whole mouth, AT = anterior tongue, NID = nasoincisor duct, FOL = foliate papillae, SP = soft palate, CV = circumvallate papilla, RM = retromolar mucosa, SLO = sublingual organ.

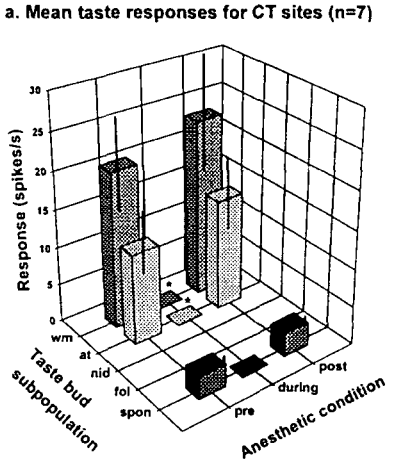
stimulation of the posterior tongue, 12 to whole mouth and foliate stimulation and another seven to circumvallate stimulation. The receptive fields for all sites are summarized in Table 1. Only three of the 59 sites responded to both anterior and posterior tongue stimulation.

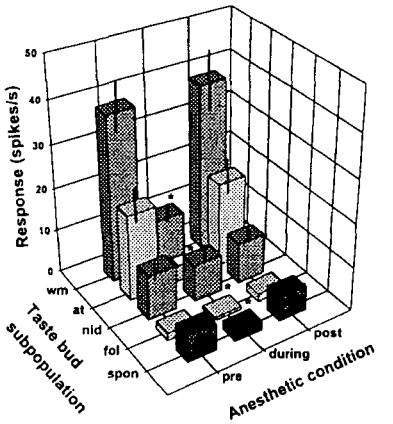
Taste responses before CTN anesthesia

Similar to previous investigations (Travers *et al.*, 1986; Travers and Norgren, 1995), we noted differences in gustatory responses and spontaneous rate at recording sites with different peripheral inputs. Because this analysis compared responses at different recording sites and multi-unit activity reflects the number of recorded units as well as their firing rate, only single-unit responses were used for these analyses. Prior to anesthesia, the mean whole mouth gustatory responses for CT and CT-mixed sites were similar (20.6 ± 6.4 versus 21.2 ± 5.0 spikes/s respectively, $P = 0.94$, *t*-test) but responses at CT-mixed sites were significantly larger that those of the non-CT group (10.0 ± 2.7 spikes/s, $P = 0.05$, *t*-test for non-CT versus CT-mixed). A comparable pattern was observed for spontaneous activities before CTN anesthesia. The average spontaneous activities were 3.0 ± 1.2 spikes/s for the CT sites, 3.1 ± 1.2 spikes/s for the CT-mixed sites and 0.29 ± 0.07 spikes/s for the non-CT sites (CT versus CT-mixed, $P = 0.96$; non-CT versus CT-mixed, $P = 0.038$, *t*-tests). In summary, single units in the CT and CT-mixed groups had comparable rates of spontaneous and evoked activity, but non-CT single units were less active.

The effect of CTN anesthesia on taste responses: averaged responses

In the following analyses we combine multi- and single-unit data to compare responses before, during and after CTN anesthesia unless otherwise noted. The mean responses (multi- and single-unit sites combined) for whole mouth, anterior tongue, nasoincisor duct and foliate papillae stimulation elicited before, during and after recovery from anesthesia are depicted in Figure 4a–c for the CT, CT-mixed and non-CT sites not tested for circumvallate stimulation respectively. Whole mouth and circumvallate responses for non-CT sites responsive to circumvallate stimulation (non-CT_{cv}) appear in Figure 5. Whole mouth and anterior tongue responses for CT sites (Figure 4a) remained stable before and after recovery from CTN anesthesia ($P > 0.1$ for both responses). CTN anesthesia abolished whole mouth and anterior tongue responses for CT sites ($P < 0.05$ for both responses). Similarly, whole mouth and anterior tongue responses for CT-mixed sites (Figure 4b) remained stable in the unanesthetized state ($P > 0.1$ for both responses) and decreased during anesthesia ($P < 0.001$ for both). Nasoincisor duct responses did not change during CTN anesthesia for CT-mixed sites (main effects: $P > 0.05$). Somewhat surprisingly, whole mouth responses for the non-CT sites (Figure 4c) were significantly decreased during





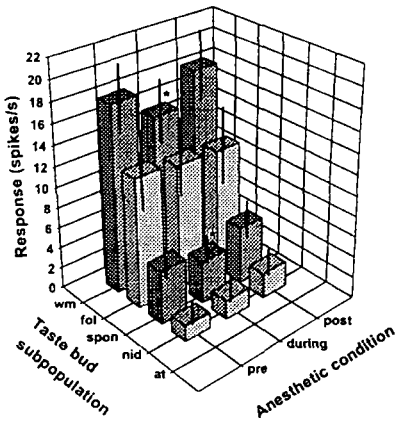


Figure 4 **(a-c)** Mean taste responses (± SEM) for each of the three categories of recording sites for each taste bud subpopulation and anesthetic condition. The abbreviations are as follows for the taste bud subpopulations: wm = whole mouth, at = anterior tongue, nid = nasoincisor duct, fol = foliate papillae, spon = spontaneous rate. Responses appear for pre-, during and post-anesthesia conditions. Note the difference in scales used for each graph and the different orientations used to differentiate each column more clearly. Statistically significant differences are shown by asterisks for anesthetized versus unanesthetized responses when $P < 0.05$ as determined by ANOVAs.

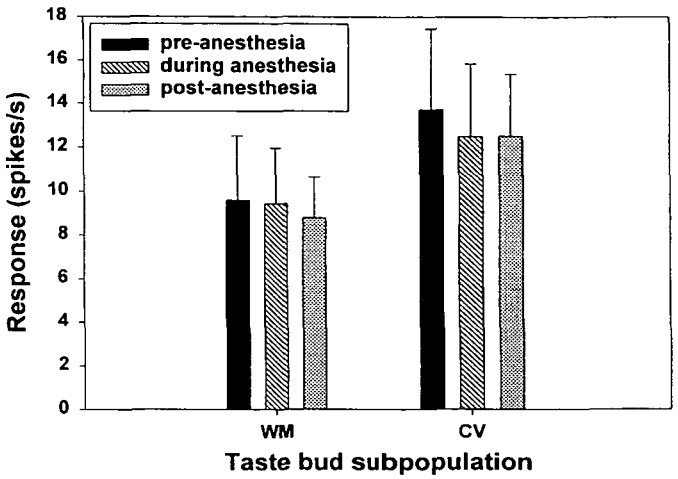


Figure 5 Mean taste responses for the seven circumvallate papilla responsive sites. The whole mouth (WM)- and circumvallate papilla (CV)-elicited responses are shown with standard error bars before (pre-), during and after recovery (post-) from CTN anesthesia. No significant changes were found as determined by ANOVAs.

anesthesia ($P < 0.01$), but remained stable in the unanesthetized state ($P > 0.1$). The whole mouth decrement at non-CT sites, however, was not reflected in nasoincisor duct- or foliate papillae-elicited responses for these sites (main effects for both: $P > 0.1$). With minor exceptions, these results were the same when the analysis was restricted to single units in the CT-mixed and non-CT groups. For non-CT_{cv} sites ($n = 7$) (Figure 5), there were no main effects of anesthesia for either the whole mouth or CV responses (for both $P > 0.1$). In summary, except for the small decrease in the whole mouth response at non-CT sites, the only effect of CTN anesthesia was the abolition of activity evoked by gustatory stimulation of the anterior tongue, which was reflected in the abolition or decrement of whole mouth responses at CT and CT-mixed sites respectively. Contrary to our original hypothesis, no increases in average responsiveness occurred during anesthesia.

The effect of CTN anesthesia on taste responses: an individual basis

As discussed in Materials and methods, we analyzed individual as well as averaged responses to avoid missing effects that might occur only for a subset of sites. The results of the individual analysis are summarized in Table 2, which lists the number of suprathreshold taste responses from multi- and single-unit sites that increased, decreased or did not change during CTN anesthesia, categorized by taste bud subpopulation. With this analysis, six of 59 (10.2%) sites exhibited response increases during anesthesia that exceeded the criteria for a reliable change, i.e. they were twice as large as the standard deviation of the responses in the unanesthetized state. Figure 6a depicts mean responses in the unanesthetized state (± SDs) compared with the

Table 2 Changes in individual taste responses for each taste bud subpopulation during CTN anesthesia

Taste bud subpopulation	Increased response	Decreased response	No change in response	Total
WM	1(2)	37(66)	18(32)	56
AT	0	29(97)	1(3)[a]	30
NID	4(15)	7(26)	16(59)	27
FOL	1(6)	3(19)	12(75)	16
CV	0	1(14)	6(86)	7
SP	0	1(25)	3(75)	4
SLO	0	1(100)	0	1
Total	6(4)	79(56)	56(40)	141

The following abbreviations are used: WM = whole mouth, AT = anterior tongue, NID = nasoincisor duct, FOL = foliate papillae, CV = circumvallate papilla, SP = soft palate, SLO = sublingual organ. The numbers in parantheses indicate the percent of cases that respond in a particular way for the total number of responses for each taste bud subpopulation.

[a]The during CTN anesthesia response was 0 spikes/s, but due to the large variability in responses in the unanesthetized state, the lower value of the criterion was a negative number. Therefore, using this type of analysis, we could not conclude that the response was decreased even though it was 0 spikes/s.

responses that occurred during anesthesia, for these six sites. In general, the increases were small and most frequently (4/6) involved nasoincisor duct responses. When responses were averaged across these six sites, responses in the unanesthetized state (pre- versus post-anesthesia) changed by 8.9%, compared with an increase of 32.9% during anesthesia. The responses for one neuron with an augmented response are shown in more detail in Figure 6b. Prior to and after recovery from anesthesia, this neuron responded to whole mouth and nasoincisor duct, but not anterior tongue or foliate papillae stimulation. The responses to palatal stimulation before and after recovery from anesthesia were nearly identical, 14.9 versus 14.3 spikes/s, but during anesthesia the response increased by 20.5% to 17.6 spikes/s. The small magnitude of this increase seems even less impressive since there is not a similar increase in the whole mouth response, despite that fact that the cell apparently received no CTN input. The largest absolute increase in response during CTN anesthesia was for a foliate papillae response which increased by 10 spikes/s (extreme right example in Figure 6a).

As predicted by the averaged data, rather than increasing during CTN anesthesia, most responses decreased (79/141, 56%) or did not change (56/141, 40%). That the effects of anesthesia were consistent and our criterion sensitive is supported by the fact that nearly all responses elicited by stimulating CTN-innervated receptor subpopulations—i.e. 29/30 anterior tongue responses and the single sublingual response—met the criterion for a decrease. In contrast to the increases just discussed, the average decrement for the

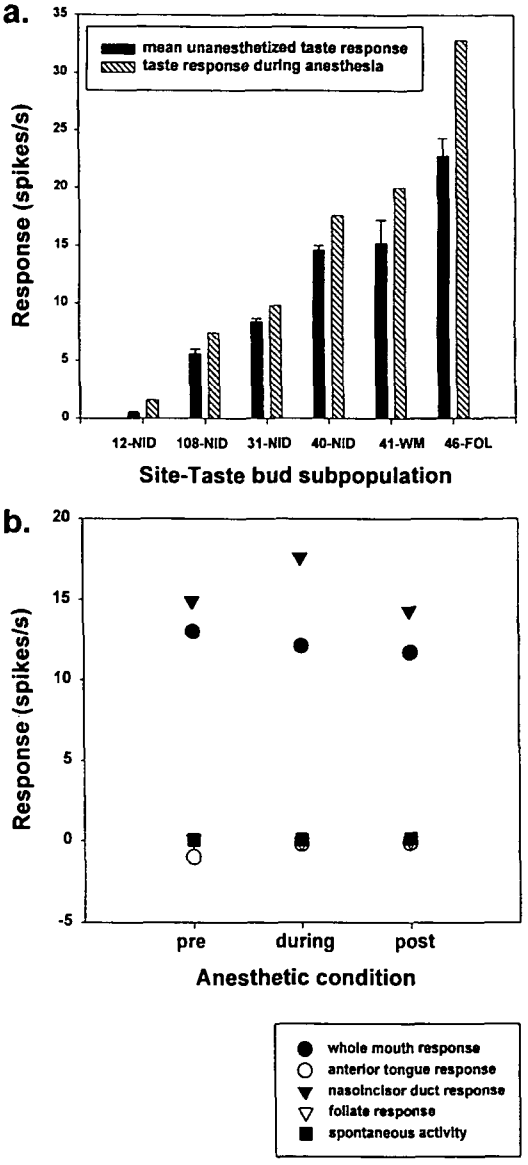


Figure 6 **(a)** Six individual sites which revealed increased taste responses during CTN anesthesia. The single response in the anesthetized state is compared with the mean (± SD) of the responses before and after recovery from anesthesia (pre- and post-anesthesia responses). The receptive field for the response which increased is listed next to the site number. **(b)** An individual site which displayed an increased nasoincisor duct taste response during anesthesia, but the whole mouth response remained the same.

anterior tongue responses was nearly complete (= 99.5%). The consistency of this anesthetic effect is apparent in the individual whole mouth responses depicted in Figure 2. Anesthetizing the CTN abolished responses to gustatory stimulation of the whole mouth for all CT sites and produced decrements in the whole mouth responses for nearly all of the CT-mixed sites. In addition to these expected decrements, it was interesting that response decrements were also observed for receptor subpopulations innervated solely or principally by a nerve other than the CTN.

Not surprisingly these decrements were smaller and less frequent. Thus, 7/23 nasoincisor, 1/7 circumvallate and 1/4 soft palate responses exhibited decrements during CTN anesthesia (= 41.6%). Even for foliate-elicited responses, decrements were not common. These papillae are principally innervated by the glossopharyngeal nerve but also receive minor CTN innervation. However, only 3/16 foliate responses exhibited decrements during anesthesia, and they were also small (= 18.6%).

The effect of CTN anesthesia on spontaneous activity

In addition to abolishing responses evoked by anterior tongue stimulation, another salient effect of CTN anesthesia was a decrease in spontaneous activity. This effect occurred for all types of recording sites but was more pronounced for some (Figure 4). Relative to the average spontaneous activity prior to anesthesia, spontaneous activity during CTN anesthesia decreased by 100% for CT, 65.2% for CT-mixed and 13.1% for non-CT sites. The average spontaneous activity for the seven cells in the CT group dropped from 3.0 to 0.0 spikes/s, although this decrease only approached significance ($P = 0.068$), probably due to a floor effect and the small number of cells. Although decreases in spontaneous activity for the CT-mixed and non-CT multi- and single-unit sites were smaller, both were significant ($P < 0.001$, $P = 0.05$ respectively). The ten single units in the CT-mixed group also reflected the overall decrease in spontaneous activity during CTN anesthesia ($P = 0.05$), although this was not true of the non-CT single units. In addition, no change during anesthesia was noted for the seven sites responsive to circumvallate stimulation.

These effects on spontaneous activity were also evident when individual multi- and single-unit recording sites were analyzed. Most sites exhibited decreases in spontaneous firing during CTN anesthesia (29/59, 49%) or did not change (27/59, 46%), whereas only a few exhibited increases (3/59, 5%). Decrements occurred most frequently (6/7, 86%) for CT sites and spontaneous activity was nearly abolished (= 98.1%). Many CT-mixed sites (19/26, 73%) also had decrements in spontaneous activity during anesthesia and these were fairly large (= 68.1%). Fewer non-CT sites (4/26, 15%) decreased and the change was smaller but notable (= 22.5%).

Relative taste responses

The relative response as a measure of quantifying the present data was conceived because of the widespread decrease in spontaneous activity that occurred during CTN anesthesia. The relative response was calculated as the standard gustatory response (net-evoked activity) divided by spontaneous activity. Except for CT sites, which were virtually silent during anesthesia, relative responses were calculated for all CT-mixed and non-CT multi- and single-unit sites, except in the few cases (4/26 CT-mixed and 3/26 non-CT sites) where this was not possible because

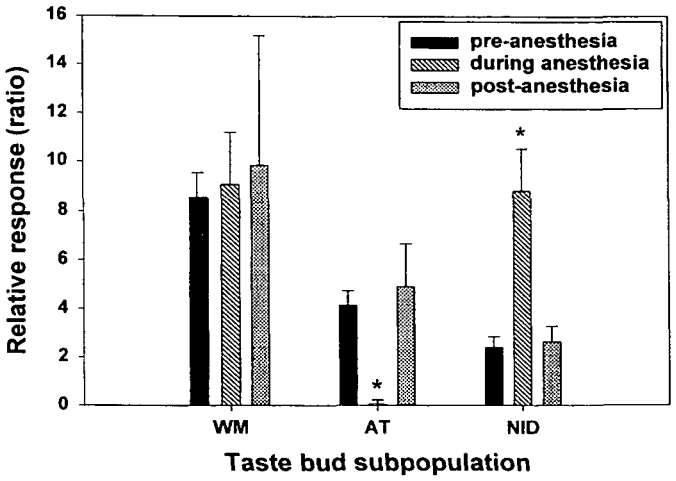


Figure 7 Mean relative taste responses for sites in the CT-mixed group. The relative response is defined as the standard taste response divided by spontaneous activity for a given response. The whole mouth (WM)-, anterior tongue (AT)- and nasoincisor duct (NID)-elicited responses are shown to highlight the constancy of the whole mouth response during CTN anesthesia. This is apparently due to the increase in relative NID response versus the decrease in AT response. The statistically significant changes during CTN anesthesia as determined by ANOVAs are indicated by an asterisk.

of the total lack of spontaneous activity. Similar to what was apparent for responses calculated in the standard fashion, anterior tongue relative responses at CT-mixed sites decreased during anesthesia (anesthetized versus unanesthetized, $P < 0.001$). However, the effects of anesthesia were different for whole mouth and nasoincisor duct responses, calculated using the relative (Figure 7) versus the standard (Figure 4b) measures. In contrast to the decrease apparent for standard whole mouth responses at these sites (Figure 4b), the relative response did not change during anesthesia (main effect: $P > 0.1$, Figure 7). Most strikingly, nasoincisor duct relative responses actually increased in the anesthetized state (anesthetized versus unanesthetized, $P < 0.005$, Figure 7), in contrast to the lack of an anesthetic effect when responses were calculated using the standard measure (Figure 4b). To illustrate this point further, Figure 8 displays histograms of neural activity (spikes/s) before, during and after recovery from anesthesia for a cell in the CT-mixed group. During anesthesia there is an obvious decline in whole mouth taste response and a complete abolishment of anterior tongue response. However, the evoked nasoincisor duct taste response is unaltered during anesthesia while the spontaneous activity decreased profoundly. Therefore, the remaining nasoincisor duct response relative to a decreased spontaneous activity has increased. This was a common finding for individual CT-mixed sites and is supported by the mean data (Figure 7). In contrast to what was found for CT-mixed sites, analysis of relative responses for the non-CT and non-CT_{cv} sites revealed a pattern of results very similar to those obtained

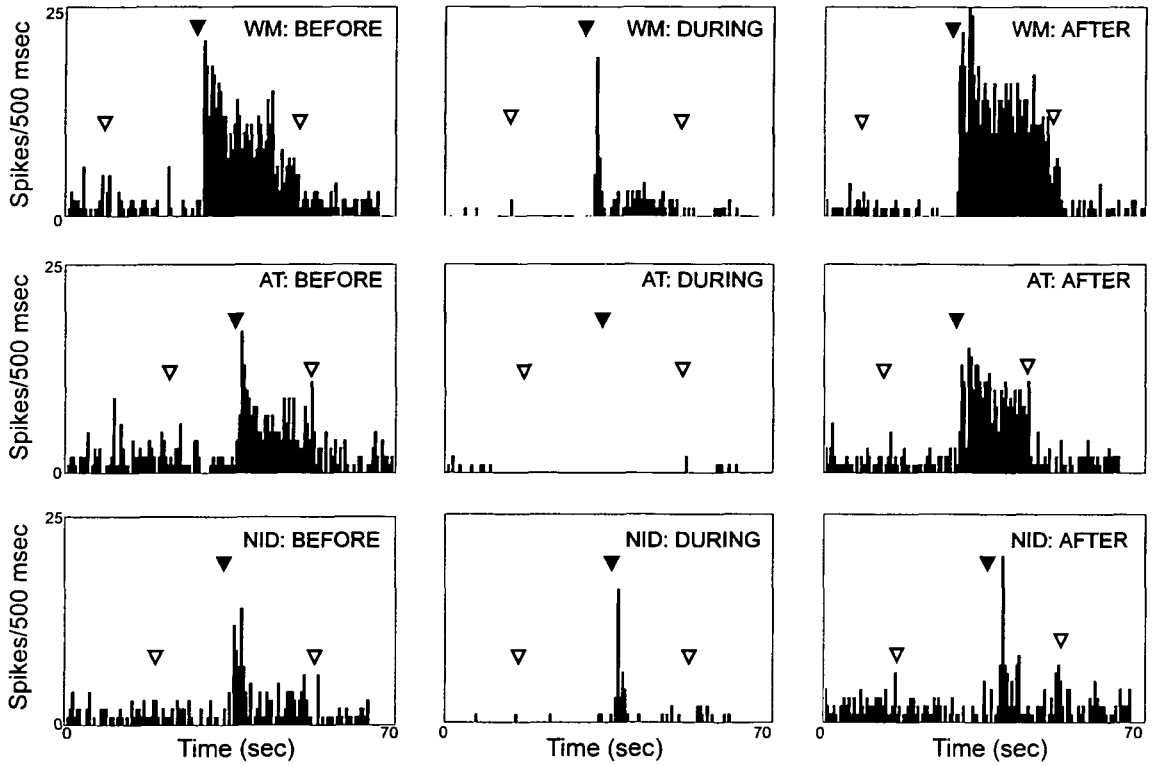


Figure 8 Peristimulus time histograms for a representative CT-mixed cell. Filled triangles depict the onset of taste stimulation and open triangles indicate water onset. Note the decreases in net spikes elicited by whole mouth and anterior tongue stimulation, as well as the decrease in spontaneous rate. However, the net nasoincisor duct response is unaltered.

when standard responses were analyzed. Anesthesia did not affect averaged relative responses for these sites.

Discussion

Contrary to our original hypothesis, the present investigation did not reveal an average increase during CTN anesthesia in net firing evoked by stimulation of any taste bud subpopulation for any group of recording sites. Instead, anterior tongue responses were abolished and whole mouth responses abolished or diminished, respect- ively, at CT and CT-mixed sites, demonstrating the efficacy of our anesthetization procedure. Although mean responses were not enhanced, there were six individual sites where responses increased during anesthesia. Specifically, six sites exhibited responses that exceeded the average response in the unanesthetized state by two standard deviations. Although there were insufficient data at individual recording sites for statistical analysis, this criterion is similar to using a 95% confidence interval, making it reasonable to suggest that these increases do not represent random variability. Although apparently reliable, increases occurred less frequently than predicted. It is, of course, possible that more subtle excitatory effects of CTN anesthesia were missed.

In addition to their infrequent occurrence, increases were small and the receptive fields of the augmented responses were not entirely consistent with our original hypothesis. We predicted that responses arising from taste bud subpopulations innervated by the glossopharyngeal nerve would increase following CTN anesthesia. However, of the 17 foliate papillae and seven circumvallate-responsive sites tested, only one with a foliate input increased during anesthesia. Of the five remaining sites where response increases occurred, four were CT-mixed sites, and the enhanced responses occurred following nasoincisor duct stimulation. Therefore, disinhibition of taste responses actually predominated for gustatory signals arising from taste buds innervated by a branch of the VIIth rather than the IXth nerve. A complication of interpreting the negative data for IXth-nerve-mediated responses arises for foliate papillae stimulation. That is, foliate taste buds receive some CTN innervation, mainly to those receptors in the anterior two folds of these papillae (Whiteside, 1927; Miller *et al.*, 1978; reviewed in Travers and Nicklas, 1990). Thus, it is conceivable that response increases occurred for the glossopharyngeal component of the foliate response but were masked by decrements occurring for the CTN component. However, such an explanation seems unlikely given the minimal amount of CTN-foliate innervation. There is no complication for interpreting the lack of increased IXth-nerve-mediated responses for circumvallate

stimulation, since these receptors are entirely innervated by the IXth nerve (Whiteside, 1927).

Comparison with previous neurophysiological studies

The increases in gustatory responsiveness that occurred following CTN anesthesia appear to agree with the results of Halpern and Nelson (1965) in their magnitude, although they seem to differ in their frequency of occurrence. At 'composite' recording sites, i.e. those sites responsive to stimulation of the anterior tongue and to receptors outside the tongue chamber, Halpern and Nelson found that instilling 3% mepivicaine in the ipsilateral external auditory meatus abolished anterior tongue responses but that responses evoked by stimulating receptors outside the chamber 'tended to increase'. Although the previous study was primarily qualitative, the authors quantified the response at one site before and during CTN anesthesia. Integrated responses from stimulation of receptors outside the chamber increased from 9.5 to 12 units during anesthesia—a 20.8% increase. The increases in response magnitudes that we observed were in a similar range, however the frequency with which we encountered them appears much lower. The previous authors apparently found consistent increases in five animals (we are assuming that this is 5/5 animals since the authors did not report the total number of animals tested in this way). In contrast, we observed that anesthetic-induced increments occurred only at a minority (3/24) of recording sites that appear analogous to theirs; i.e. most of our CT-mixed sites. Indeed, at seven of our sites we found decrements in non-anterior tongue responses. The reason for the lower proportion of anesthetic-induced increases in our study is not clear, although we tested a larger sample of recording sites and compared responses in the anesthetized state to those both prior to and after recovery from anesthesia. Although the previous authors attributed response increases to posterior tongue stimulation, we found that responses following palatal stimulation increased more frequently during anesthesia. However, the interpretation by the previous authors is probably merely a result of the fact that palatal taste buds had not been described at that time, in combination with the non-specific stimulation techniques used.

The effects of chorda tympani anesthesia or acute nerve cuts have also been studied in the major synaptic target of NST efferents, the parabrachial nucleus (PBN) (Norgren and Pfaffmann, 1975; Miyaoka *et al.*, 1997). In agreement with the present study, neither investigation provides evidence for release of inhibition, although the posterior tongue was not specifically stimulated in either case. Norgren and Pfaffmann (1975) recorded from single PBN neurons before, during and after recovery from CTN anesthesia with 2% lidocaine. Ten cells responded to stimulation of receptors outside an anterior tongue chamber; of these, seven responses persisted during CTN anesthesia. However, none of the remaining responses increased. Instead, most declined. Similarly, a recent study used an across-animal design to compare responses in a sizeable sample of PBN neurons in CTN denervated and intact animals. Quinine-elicited taste responses did not change between the two groups, suggesting a lack of compensatory change (Miyaoka *et al.*, 1997).

The non-additive effects of simultaneous stimulation have also been cited as evidence for opposing peripheral interactions in the first-order gustatory relay (NST) (Sweazey and Smith, 1987; Miller and Bartoshuk, 1991; Lehman *et al.*, 1995; Grabauskas and Bradley, 1996). In the hamster NST (Sweazey and Smith, 1987), 11 single neurons responsive to receptors on the anterior tongue and outside the anterior tongue chamber were studied. The response to individually stimulating each region was excitatory, but simultaneous stimulation usually produced responses the same as or slightly greater than the largest individual response. Although such effects have been interpreted as excitatory/inhibitory interactions (Sweazey and Smith, 1987; Miller and Bartoshuk, 1991; Lehman *et al.*, 1995; Grabauskas and Bradley, 1996), lack of summation is not equivalent to inhibition. A recent *in vitro* slice preparation similarly reported that stimulation of the solitary tract at sites consisting primarily of VIIth or IXth nerve fibers usually resulted in both sites evoking excitatory post-synaptic potentials (Grabauskas and Bradley, 1996). Thus, interactions between peripheral gustatory influences are ubiquitous in NST but do not appear to be predominantly characterized by opposing inputs from the VIIth versus IXth nerves.

Relation to psychophysical effects of CTN anesthesia or section

A more complex problem exists in attempting to reconcile our results with human psychophysical data and clinical observations which provide evidence for compensatory mechanisms following CTN anesthesia or damage. Unless subjected to spatial testing, CTN anesthesia or damage does not produce notable changes in perceived intensity (Rice, 1963; Bull, 1965; reviewed in Miller and Bartoshuk, 1991). Although experiments in rodents have demonstrated important deficits in threshold detection (Spector *et al.*, 1990) or qualitative discrimination (Spector and Grill, 1992) for specific combinations of nerve cuts and tastants, the resistance of the gustatory system to partial denervation is a salient feature of its organization in the rat as well. Indeed, one intensive aspect of gustatory-guided behavior, namely, concentration-dependent increases or decreases in lick rate, appear mostly resistant to partial gustatory denervation (Spector *et al.*, 1993; St John *et al.*, 1994). A compensatory increase in responses to stimulation of residual taste buds is a simple hypothesis which would explain this resilience. Indeed, Lehman *et al.* (1995) provided direct psychophysical support for such a mechanism in humans, demonstrating that CTN anesthesia induced an increase in the perceived

intensity of quinine applied to the circumvallate papillae. However, we did not observe an increase in circumvallate-elicited responses at the level of the first-order gustatory relay in the rat. This discrepancy could be due to a species difference, although such a possibility is difficult to address.

As discussed above, data from the other brainstem taste nucleus, the PBN, also provide little evidence for response increases following denervation or anesthesia. However, in other sensory systems the most striking evidence for anesthesia- or denervation-induced plasticity is for the forebrain, particularly the cortex, although smaller changes have been reported for lower levels (reviewed in Kaas, 1991). Indeed, it is interesting that the psychophysical effects for circumvallate stimulation were observed to be greater contralateral to CTN anesthesia, although bilateral changes were observed (Lehman *et al.*, 1995). The ascending gustatory system is primarily ipsilateral, but some bilateral ascending projections occur at levels rostral to NST and there are opportunities for bilateral interactions via descending projections, e.g. from the cortex to NST (Norgren and Grill, 1976; van der Kooy *et al.*, 1984; reviewed in Norgren, 1993). Thus, compensatory changes originating or occurring at forebrain levels are possible. If cortical influences are necessary for compensatory changes to occur in the NST, they could have been dampened in the present study. In this study animals were anesthetized with urethane and sodium pentobarbital. The latter agent, in particular, decreases cortical activity (Clark and Rosner, 1973), which in turn can alter NST taste cell responsiveness (Hayama *et al.*, 1985; Nakamura and Norgren, 1991). Although we attempted to minimize the total amount of pentobarbital by combining it with urethane, cortical suppression of activity undoubtedly occurred. Thus, it might be fruitful to re-examine CTN anesthetic effects in NST in a chronic preparation or one which uses a different anesthetic regimen.

An increase in signal-to-noise ratio

Although there was minimal evidence for compensatory increases when responses were quantified using net firing rate, a different perspective is suggested by our alternative 'relative' response measure, which quantifies evoked activity on a proportional basis. Specifically, at recording sites receiving both anterior tongue and nasoincisor duct inputs, anesthesia eliminated lingual responses and reduced whole mouth responses but left net nasoincisor duct-evoked responses unaltered (see Figure 4b). At these sites, CTN anesthesia also reduced spontaneous firing rate by more than 50%. As a consequence, although the relative measure still revealed anterior tongue responses to be eliminated, average nasoincisor duct responses increased by threefold and whole mouth taste responses were unaltered (Figure 7). Thus, if information about stimulus intensity is conveyed by proportional increases in neural activity, intensity may be conserved for these recording sites. A similar suggestion has been made with regard to the action of dopamine in the frog olfactory bulb. Dopamine reduced the spontaneous rate of mitral cells but did not alter olfactory-evoked responses, prompting the hypothesis that this neuromodulator increased the 'signal-to-noise' ratio via its effect on spontaneous activity (Duchamp-Viret *et al.*, 1997). We suggest that the reduction in spontaneous rate induced by CTN anesthesia might have a similar function in increasing the signal-to-noise ratio of nasoincisor duct-evoked gustatory responses in the NST. It should be kept in mind, however, that, if this type of compensation does occur, it is limited to a specific subset of neurons—those receiving convergent inputs from the CTN and other peripheral sources.

Aside from these considerations, CTN anesthetic effects on spontaneous rate are interesting in their own right. Under our experimental conditions, nearly all the spontaneous activity in neurons that respond only to anterior tongue stimulation can be blocked by CTN anesthesia, suggesting that peripheral inputs contribute importantly to baseline activity. Since many CTN afferents are sensitive to stimulation with sodium salts (Frank *et al.*, 1983), activation by salivary sodium ions may contribute to the spontaneous activity in central neurons receiving inputs from this source. A relatively high spontaneous activity in anterior-tongue responsive fibers and their central counterparts may serve an important function in allowing the system to respond to both increases and decreases in sodium concentration, from either saliva or external sources.

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