Altered Taste Responses in Adult NST After Neonatal Chorda Tympani Denervation

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Dinkins, Mark E. and Susan P. Travers. Altered taste responses in adult NST after neonatal chorda tympani denervation. J. Neurophysiol. 82: 2565–2578, 1999. Anatomic and behavioral changes have been observed in the taste system after peripheral deafferentation, but their physiological consequences remain unknown. Interestingly, a recent behavioral study suggested that peripheral denervation could induce central plasticity. After neonatal chorda tympani (CT) transection, adult rats demonstrated a marked preference for a normally avoided salt, NH4Cl. In the present study, taste responses were recorded from the nucleus of the solitary tract (NST) in similarly CT-denervated rats to investigate a physiological basis for this behavioral phenomenon. We hypothesized that alterations in functional connectivity of remaining afferent nerves might underlie the behavioral change. Specifically, if NST neurons formerly activated by sodium-selective CT fibers were instead driven by more broadly tuned glossopharyngeal (GL) afferents, neural coding of salt responses would be altered. Such a change should be accompanied by a shift in orotopic representation and increased NH4Cl responses. This hypothesis was not supported. After CT denervation, orotopy was unaltered, NH4Cl responsiveness declined, and no other changes occurred that could simply explain the behavioral effects. Indeed, the most pronounced consequence of CT denervation was a 68% reduction in NaCl responses, supporting previous evidence for a critical role of this nerve in coding sodium salts. In addition, we found “reorganizational” changes similar to, albeit smaller than, those observed in other sensory systems after deafferentation. There was a trend for increased responses elicited by stimulation of receptor subpopulations innervated by the GL and greater superficial petrosal nerves. In addition, the spontaneous rate of nasoincisor duct-responsive cells increased significantly. This effect on spontaneous rate is opposite to that produced by CT anesthesia, suggesting that acute versus chronic denervation may affect central taste neurons differently. In conclusion, the taste system at the medullary level seems more resistant to large-scale plasticity than other sensory systems, but nevertheless reacts to lost afferent input. Because the most robust plastic changes have been documented at cortical levels in other sensory pathways, the substrate for the behavioral effect of neonatal CT transection may be located more centrally in the gustatory system.

INTRODUCTION

Central neural reorganization after peripheral deafferentation has been well documented in visual, somatosensory, and auditory systems (reviewed in Buonomano and Merzenich 1998; Donoghue 1995; Weinberger 1995). By comparison, little is known of the central effects of peripheral deafferentation in the gustatory system. Recent behavioral evidence, however, suggests that neonatal gustatory denervation may cause central reorganization. Adult rats that received chorda tympani nerve (CT) transection at 10 days of age, but not those transected in adulthood, demonstrated a striking behavioral preference for ammonium chloride at normally avoided concentrations (Sollars and Bernstein 1996). Sollars and Bernstein proposed that this new-found preference might be due to reorganization of remaining afferent input in the first-order taste relay, the nucleus of the solitary tract (NST). Indeed, reorganizational changes in other sensory systems are very robust in younger animals (e.g., Kalaska and Pomerantz 1979; reviewed in Kaas et al. 1983; O’Leary et al. 1994; Wilson and Kitchener 1996). For example, restricted lesions of the vibrissal pad on the day of birth are associated with an expansion and reorientation of afferents supplying undamaged regions of the whisker pad in the trigeminal nucleus interpolaris (Renehan et al. 1994).

Peripheral deafferentation has anatomic consequences in the NST of developing and adult rodents. Anterior tongue cautery in 2-day-old rats causes CT terminal field volume to decrease (Lasiter and Kachele 1990); adult CT transection results in transganglionic degeneration (Whitehead et al. 1995). Thus CT transection eliminates afferent activity and causes the loss of synaptic sites, which may allow central reorganization, namely increased efficacy of spared afferents (Merzenich et al. 1983a, 1988). Developmental factors suggest that neonatal CT denervation could affect glossopharyngeal nerve (GL) inputs. Chorda tympani fibers begin to terminate in NST prenatally, but GL termination commences 9–10 days postnatally (Lasiter 1992, 1993), coincident with the timing of behaviorally effective CT transections (Sollars and Bernstein 1996). Interestingly, the CT and GL respond differently to salts. Although both are activated by NH4Cl and the preferred salt, NaCl, some single CT fibers respond selectively to sodium salts (Boudreau et al. 1983; Dahl et al. 1997; Frank et al. 1983; Hill et al. 1982, 1983), whereas GL fibers are much less selective (Frank 1991). Together, these considerations suggest the hypothesis that neonatal CT transection enhances GL input in the CT field and switches the input of some NST cells from sodium-selective CT fibers to broadly tuned GL afferents, leading to a change in central salt coding with behavioral consequences.

These arguments for the reorganization of GL responses, however, certainly do not exclude the possibility of other alterations. In particular, although little is known about its development or single-fiber response profiles, reorganization of the greater superficial petrosal nerve, which innervates palatal taste receptors, also seems feasible, in this case on the basis of its termination pattern. In contrast with the GL, GSP terminations demonstrate a high degree of overlap with those from
the CT (Hamilton and Norgren 1984), and many individual NST neurons receive convergent input from both the anterior tongue and palate (Travers et al. 1986; Travers and Norgren 1991, 1995). Similar to the CT and GL, whole nerve recordings from this nerve demonstrate a robust responsiveness to NH₄Cl (Nejad 1986; Sollars and Hill 1998), suggesting that palatal stimulation could also drive altered behavioral responses to this chemical.

The objective of the current study was to determine whether neonatal CT transection leads to central reorganization in the adult NST, as reflected in neurophysiological gustatory responses. Specifically, to find correlates of the behavioral effects, we investigated whether GL-mediated posterior tongue responses shifted anteriorly to NST locations where CT-mediated anterior tongue responses are typically found (Travers and Norgren 1995) and whether the number or magnitude of posterior tongue or NH₄Cl-elicited responses increased. In addition, we examined whether other forms of plasticity were evident: whether there were increases in spontaneous rate, responsiveness to other chemicals or taste bud subpopulations, or whether novel receptive field organizations or chemosensitive neuron types appeared. Although not all these possibilities would necessarily explain the behavioral changes noted by Sollars and Bernstein (1996), they would be consistent with denervation-induced changes in other sensory systems (e.g., Gilbert and Wiesel 1992; Merzenich et al. 1983a; Pons et al. 1991; Schwaber et al. 1993). To assess whether an orotopical shift had occurred, results of multi- and single-unit recordings were combined to systematically map a large area of the NST. Changes in neural responsiveness were determined by recording from individual neurons.

METHODS

Subjects and anesthesia

Twenty-two male and eight female Long-Evans rats were used, 5 for multiunit mapping and the remaining 25 for single cell recording (232–498 g; males, mean = 406 g and females, mean = 262 g). Procedures for multi- and single-unit recording were similar unless noted otherwise. Rats underwent two surgical procedures. For CT transection at 10 days of age, they were anesthetized with a mixture of ketamine and xylazine (3.7 and 0.74 mg/kg ip, respectively). For neurophysiological tests at adult age (82 ± 2.8; range, 57–115 days), rats were anesthetized with pentobarbital sodium alone (Nembutal, 50 mg/kg ip) or in combination with ethyl carbamate (urethan, 1 gm/kg ip; Nembutal, 25 mg/kg ip). Supplemental doses of Nembutal were given as needed. Animal procedures were approved by the Ohio State University’s Institutional Laboratory Animal Care and Use Committee.

Chorda tympani transection

At 10 days of age, rats underwent CT transection similar to that of Sollars and Bernstein (1996), except that it was unilateral. Unilateral cuts were made to allow recording from comparable sites in rostral NST (rNST) in both the transected and intact state. It was particularly critical to compare responses in the rostral pole of the NST, i.e., the region that receives CT input. Our strategy of recording from the innervated side of a particular rat and switching to symmetric locations on the cut side controlled for the possibility that the denervated area of the rNST could be silent. Moreover, because primary afferent gustatory input to rNST is almost entirely ipsilateral (Hamilton and Norgren 1984), it seemed likely that transection-induced plasticity would be apparent in unilaterally transected animals and the contralateral side should serve as a reasonable control. Although contralateral changes have been noted in other sensory systems following ipsilateral perturbations, ipsilateral changes are far more pronounced (e.g., Takemura et al. 1998). Importantly, in the present study, the topographic organization, as well as the mean spontaneous rate on the contralateral side were not different from those observed in another recent study in our lab that used a similar design (r = 0.24, df = 56.6, P = 0.81) (Dinkins and Travers 1998).

For CT transection, a midline ventral neck incision was made. Blunt dissection was performed to trace the lingual nerve to its anastomoses with the CT (see Richter 1956, for illustration). Once the CT was visualized, its distal end was teased from the lingual nerve, and the remaining proximal portion was removed. Typically, a 5- to 7-mm piece of CT was removed, ensuring that regeneration would be very unlikely. The wound was closed and sutured. Pups were returned to the dam after ~2 h and monitored daily for a 1- to 2-wk period. There were no instances of maternal rejection, and it was common to observe the pups suckling from the dam immediately after their return. One rat pup, which did not gain weight during the first 3 days postop, was removed from the study.

Neurophysiological surgical preparation

Surgical preparatory procedures for acute neurophysiological recording were similar to those previously described (Travers et al. 1986; Travers and Norgren 1995). Briefly, adult transected rats were stabilized on a stereotaxic apparatus using a mouthpiece and atrumatic earbars (Kopf Instruments, Tujunga, CA). The rat’s head was leveled with respect to lambda and bregma landmarks on the skull in the horizontal plane. A headholder device was fastened to one earbar and secured to the skull via small bone fixation screws surrounded by methyl methacrylate. This head holder stabilized the rat’s head while eliminating the need for a mouthpiece so that one can stimulate discrete taste bud subpopulations in the oral cavity (described in Travers et al. 1986). A tracheal cannula was inserted to allow unimpeded respiration during fluid delivery and 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 transected bilaterally. Retraction sutures were placed at four sites around the oral cavity and through the tongue to allow adequate stimulation of different taste bud subpopulations (Halsell et al. 1993; Travers et al. 1986). A craniotomy was then performed posterior to lambda to access the brain for microelectrode penetration. Physiological saline was applied to the exposed area of the cerebellum.

Neurophysiological recording session

Neural responses were recorded with glass-coated tungsten microelectrodes (150–700 kΩ for multiunit mapping and 1.0–1.5 MΩ for single-cell isolation) on the CT-intact and cut sides. Neural activity was amplified and observed on an oscilloscope and audio monitor. Anterior-posterior (AP) and mediolateral (ML) coordinates relative to lambda were noted for each track. Recording sites (multi- and single-unit) were marked with electrolytic lesions made with anodal current (3 μA, 3 s, Grass stimulator) at the recording site or at a site 200–300 μm ventral to it.

MULTIUNIT MAPPING. Many tracks (10–18 per side, mean = 13.4 intact and mean = 14.6 cut side) were made to construct a detailed map of multiunit taste and tactile responsiveness in each rat. Electrode tracks were made in a systematic manner usually 200 μm apart. Typically, 2–3 tracks at different ML locations were made per AP level. Responses were determined at 50-μm intervals dorsoventrally for each track starting ventral to spontaneous activity characteristic of vestibular nuclei and ending in strong jaw stretch activity characteristic of reticular formation. The receptive field(s) for oral taste and tactile responses were classified qualitatively on the basis of a clear increase in activity using a storage oscilloscope and audio monitor. This qualitative procedure was considered sufficient, based on agree-
ment between similar qualitative and quantitative categorizations from a previous study (Dinkins and Travers 1998).

**SINGLE-CELL ISOLATION.** The CT-intact side was sampled first because it was more efficient to locate taste-responsive neurons on this side and then to use similar coordinates to locate taste-responsive neurons on the CT-cut side. Also, by using similar coordinates, similar areas of the NST were sampled on either side. Responses from single cells were recorded on VHS tapes for off-line quantitative analysis.

**Taste and tactile stimulation**

Neural responses to taste stimulation of the whole mouth, anterior tongue, nasoincisor ducts, foliate papillae, and soft palate were assessed. On occasion, the circumbulvalleate papilla was also stimulated. Taste buds on the anterior tongue are innervated by the CT, and those within the nasoincisor ducts are innervated by the greater superficial petrosal nerve, both are branches of the facial nerve. Taste buds associated with the foliate and circumvallate papillae are innervated by the lingual-tonsillar branch of the GL, and those on the soft palate are innervated by the greater superficial petrosal nerve. The testing session started by 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 ipsilateral individual receptor subpopulations were tested. For each stimulus trial, spontaneous activity was recorded for 10 s preceding stimulation, and the water, tantant, and rinse applications were of an equal duration. 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 receptor subpopulations were stimulated with sable hair brushes in a similar water-stimulation-rinse sequence. After taste stimulation the whole mouth was rinsed with water from a syringe (Travers et al. 1986; Travers and Norgren 1995). Fluid delivery was observed through an operating microscope to ensure accurate stimulus application. Single cells were also tested using the same protocol for stimulating the whole mouth and individual receptor subpopulations, with individual tastants; 0.3 M sucrose, 0.3 M NaCl, 0.3 M NH4Cl, 0.01 M HCl, and 0.003 M quinine hydrochloride. Both multi- and single-unit sites were tested for tactile responsivity using a blunt glass probe applied to the ipsilateral buccal mucosa, anterior tongue, foliate papillae, circumbulvalleate papilla, and soft and hard palate. Stimulus onset was marked with verbal comments.

**Histological reconstruction of recording sites**

For multiunit mapping, approximately half of the tracks were marked with a lesion so that reconstruction would be as accurate as possible without confusing adjacent lesions. The other half were interpolated from the closest lesion, typically 200 μm away, at the same AP level. Almost every single unit (90/94) was marked with a lesion.

After the recording session, rats received a lethal dose of pentobarbital sodium (150 mg/kg). They were perfused intracardially with physiologic saline (300–400 ml) and fixed with 10% buffered Formalin (200–300 ml). The brain was removed and stored in 10% Formalin. Several days before cutting, the brain was transferred to a 20% sucrose/10% buffered Formalin solution for cryoprotection. Brains were cut at 52 μm away, at the same AP level. Almost every single unit (90/94) was marked with a lesion.

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**Analysis of orotopic representation**

A specific question was whether GL-mediated (posterior tongue) responses on the cut side were more rostral than on the intact side. To assess gustatory orotopic organization, multiunit responses were used to supplement the single-unit recordings because multiunit recordings accomplished a more extensive, systematic map. Although our hypothesis specifically predicted a shift of posterior tongue responses, to simplify the analysis, multi- and single-unit sites were classified using a binary scheme; i.e., as anterior and/or posterior oral cavity taste responsive. The anterior tongue and nasoincisor duct are located within the anterior oral cavity (AOC), and the foliate, circumbulvalve, and soft palate are located within the posterior oral cavity (POC). The rostral NST was divided into 10 equal AP divisions, with the caudal 5 segments collapsed into one because this area was infrequently sampled. The number of sites (multi- and single-unit sites combined) that responded to AOC and/or POC taste stimulation was compared for each division. The rostral extent of GL-mediated taste responses was compared for each side. Independent t-tests were used to determine differences in mean AP and ML locations of AOC or POC taste-responsive sites within and between sides.

**Quantification of single-unit activity**

Single cells were differentiated off-line with a window discriminator using consistency of amplitude and waveform as criteria. Activity was quantified by converting action potentials to digital pulses and accumulating these in 500-ms bins in peristimulus time histograms using MII hardware and software (Modular Instruments, Southeastern, PA). Net-evoked activity was quantified with a standard response measure, defined as the number of spikes over a 10-s period during taste stimulation minus the number of spikes that occurred during the preceding water stimulation. The criteria for a suprathreshold taste response were defined as a minimum 1 spike/s increase in activity, which also had to be >2.5 times the standard deviation of the spontaneous rate (Dinkins and Travers 1998; Travers et al. 1986; Travers and Norgren 1991, 1995; Travers and Smith 1984).

**Statistical analyses of neural responses**

To determine an effect of CT transection on cell responses, comparisons between sides were made using several analyses. Initial comparisons were made between all cells recorded on the CT intact versus the cut sides. These determined whether the sides were different but did not provide much insight regarding whether differences were due to central reorganization, because certain changes would be expected based solely on removing CT input. To make more direct comparisons of responses from peripheral sources other than the CT, we compared whole mouth responses after removing cells responsive to anterior tongue stimulation on the intact side. This included removing cells specifically responsive to anterior tongue stimulation as well as anterior tongue neurons with additional convergent inputs. We also compared responses to specific stimulation of nonanterior tongue receptor subpopulations, that is, the nasoincisor duct, foliate, or soft palate.

\[ \chi^2 \] was used to determine the difference in number of AOC multi- and single-unit responses between sides and to determine differences in the proportions of neurons with various receptive fields. Mean spontaneous rates and responses to the taste mix and individual tastants were compared between sides using two-way ANOVAs fol-
RESULTS

Taste pores and fungiform papillae

There were fewer taste pores and fungiform papillae on the CT-cut side of the tongue ($t = 26.29, df = 28, P < 0.001; t = 8.39, df = 28, P < 0.001$, respectively; Fig. 1). These results confirm the efficacy of the CT transection and provide evidence that this procedure results in long-term denervation of the tongue.

Orotopic representation of multi- and single-unit taste responses

Based on multi- and single-unit activity in 30 rats, the orotopic representation of taste responses did not change after long-term CT transection. Figure 2 depicts taste responses from both the multiunit (squares) and single-unit (circles) recording experiments, classified by their responsiveness to AOC and/or POC stimulation. In the five multiunit mapping procedures, 139 tracks were made in the vicinity of the rNST, and 109 tracks passed through the nucleus. Of these, 33 taste-responsive tracks were identified on the CT-intact side and 19 on the cut side (Fig. 2). The remaining tracks were unresponsive or responsive to other types of orosensory stimulation (see next paragraph). During 25 single-unit recording preparations, responses were obtained from 94 taste-responsive cells; 51 on the CT-intact and 43 on the cut side. The locations of 78 single neurons that were histologically reconstructed and could be classified as AOC- and/or POC-responsive are also shown in Fig. 2.

Combining multi- and single-unit data, AOC taste-responsive sites were found rostral and lateral to POC taste-responsive sites on both sides (intact side, AP, $t = 6.27, df = 65, P < 0.001, ML, t = 5.68, df = 64, P < 0.001$; cut side, AP, $t = 3.76, df = 41, P = 0.001, ML, t = 3.10, df = 41, P < 0.005$). In contrast, mean AP and ML locations for AOC taste-responsive sites were not significantly different between sides (AP, $t = 1.08, df = 75, P = 0.29; ML, t = 1.31, df = 74, P = 0.19$), nor were mean locations of POC taste-responsive sites (AP, $t = 1.76, df = 31, P = 0.09; ML, t = 0.65, df = 31, P = 0.52$). Thus contrary to our hypothesis, POC taste responses were not...
represented further rostrally in the NST following neonatal transection. Furthermore, in the multunit recording experiments, we found that the orotopic representation of mechanical responses was also unaltered by neonatal transection (not depicted).

Instead of a rostral migration of POC taste responses, the rostral pole of the NST (rostral 40%) on the cut side was less responsive to taste stimulation. There were fewer sites in the rostral pole of the NST responsive to AOC stimulation on the cut side (23 vs. 46). At locations homologous to those yielding robust anterior tongue taste responses on the intact side, responses to depressing the mandible ("jaw stretch") were typically found. In fact, a higher proportion of tracks on the cut side were characterized only by responses to jaw stretch ($\chi^2 = 9.50, P < 0.005$; see large and small asterisks, Fig. 2). Five of these tracks on the cut side passed through a clear unresponsive region, 50–100 $\mu$m in depth, that was just ventral to the high-amplitude activity characteristic of the vestibular nucleus, but dorsal to responses to depressing the mandible (large asterisks, Fig. 2). The greater number of "jaw stretch only" tracks, particularly those with this dorsal, unresponsive region, implies that neonatal CT transection renders the rostral pole of the NST relatively unresponsive. Alternatively, because these observations are based on multunit activity and ventrally placed lesions, the precise location of the jaw stretch responses is unknown, and it is conceivable that they were recorded from cells in an unusual location, i.e., in NST. However, in the single-unit experiments, six jaw stretch neurons were marked with lesions and were always found ventral to or in the ventral subdivision of NST, on both the CT-intact and cut sides. Thus, although jaw stretch responses were noted more frequently on the cut side, there is no evidence that their position changed.

**General description of single cells**

The number of taste-responsive cells per side was similar (2.00/intact side and 1.83/cut side per rat; $t = 0.31$, df = 23, $P = 0.76$). Also, a similar number of taste-responsive cells were isolated per taste-responsive track (0.42 cells/track intact side vs. 0.37 cells/track cut side; $t = 0.61$, df = 21, $P = 0.55$). However, more tracks were made on the CT-cut side to find the same number of taste-responsive cells (mean = 7.5 tracks/rat on the intact side vs. mean = 10.5 tracks/rat on the cut side; $t = 3.96$, df = 23, $P = 0.001$). Therefore identifying the taste-responsive area of the NST was more difficult on the CT-cut side.

**Receptive field organization**

Although there was no evidence for orotopic reorganization, differences in the proportions of cells responsive to particular receptor subpopulations were found. Table 1 classifies cells by receptive field, and Fig. 3 graphically compares the proportions of neurons activated by each receptor subpopulation for the two sides, excluding those (few) cells that only responded to whole mouth stimulation. A high proportion of neurons on the intact side, 28/39 (~72%) responded to anterior tongue stimulation. However, on the cut side, as expected, anterior lingual stimulation was virtually ineffective. Only one cell whose response barely met criteria responded to anterior tongue stimulation. Most cells on the cut side, 19/30 (~63%) responded to stimulation of the nasoincisor duct. Contrary to our hypothesis, an increase in the incidence of GL-responsive cells was not observed ($\chi^2 = 0.21$, $P > 0.05$). However, an increase in palatal-responsive cells was found: the proportion of nasoincisor duct-responsive cells increased over twofold on the cut side ($\chi^2 = 10.14$, $P = 0.001$), and the incidence of soft-palate responsive cells increased even more markedly. Soft palate-responsive cells comprised only ~8% of the cells on the intact, but over 40% on the cut side ($\chi^2 = 12.09$, $P = 0.001$). Thus on the denervated side, palatal but not foliate responses increased to compensate for the missing anterior tongue responses.

The increase in number of palatal responses is provocative, but removing anterior tongue input dictates that other cell types increase proportionally, even without altered input. To address this possibility more directly, we reanalyzed the data without including anterior tongue responses. Interestingly, there was still a higher proportion of soft palate-responsive cells on the cut side ($\chi^2 = 4.43$, $P < 0.05$). On the other hand, the proportion of foliate-responsive cells decreased significantly ($\chi^2 = 5.22$, $P < 0.05$) and the proportion of nasoincisor duct-responsive cells was the same ($\chi^2 = 0.69$, $P > 0.05$). The selective increase in soft palate–responsive neurons suggests that afferent input from this receptor subpopulation may become more efficacious after denervation.

Although the proportions of cells responding to particular receptor subpopulations differed between sides, the proportion that received convergent input arising from separate receptor subpopulations did not ($\chi^2 = 0.361$, $P > 0.05$). Twelve of 42 (29%) cells on the intact side and 11 of 35 (31%) on the cut side responded to more than one receptor subpopulation (e.g., both the nasoincisor duct and soft palate). The remaining neurons received input from a single receptor subpopulation. Even when cells responsive to anterior tongue stimulation were removed from the analysis, the incidence of convergence still did not vary; convergent neurons comprised 21% of cells on

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Each cell is classified by its response to stimulation with taste mixture to a particular receptor subpopulation. Only cells with fully characterized receptive fields are included ($n = 77$). RF, receptive field; CT, chorda tympani nerve; AT, anterior tongue; NID, nasoincisor duct; FOL, foliate papillae; SP, soft palate; CV, circumvallate papillae; WM, whole mouth.
the intact and 29% on the cut side ($\chi^2 = 0.32$, $P > 0.05$). These results suggest that patterns of afferent input to single cells were unaltered by neonatal CT denervation.

**Altered neural responses in the NST**

The preceding analyses investigated the possibility of central reorganization by categorizing neurons according to suprathreshold responses, without regard to response magnitude. The following analyses explore the possibility of more graded changes with respect to spontaneous or evoked activity by analyzing changes in firing rates.

**SPONTANEOUS ACTIVITY.** Because previous work has shown that the spontaneous rate of NST cells that receive CT input is greater in magnitude than cells that do not (Dinkins and Travers 1998; Travers et al. 1986), a change in the characteristic spontaneous activity could suggest denervation-induced plasticity. The overall mean spontaneous rate on the CT-intact versus the cut side was lower but not statistically significant [2.08 ± 0.39 (SE) spikes/s intact vs. 2.95 ± 0.67 spikes/s cut side; $t = 1.13$, df = 68.7, $P = 0.26$]. However, when anterior tongue-responsive cells were removed to directly compare cells without CT input, the mean spontaneous rate was significantly higher for cells on the CT-cut side (2.97 ± 0.69 spikes/s cut side vs. 0.70 ± 0.26 spikes/s intact side; $t = 3.09$, df = 50.7, $P < 0.005$). This effect was explored further and found to be restricted to cells that responded best to nasoincisor duct stimulation. Cells that responded best to nasoincisor duct stimulation on the CT-intact side exhibited a much lower mean spontaneous rate than on the CT-cut side (Fig. 4; 1.68 ± 0.61 spikes/s vs. 4.75 ± 1.06 spikes/s, respectively; $t = 2.51$, df = 31, $P < 0.05$).

**TASTE RESPONSES TO MIXTURE STIMULATION.** Response magnitudes evoked by stimulating each receptor subpopulation with taste mixture for each cell on the CT-intact and cut sides are depicted in Fig. 5; mean responses appear in the insets. Across all cells, an ANOVA of the mean responses following analyses explored the possibility of more graded changes in the character of spontaneous activity could suggest denervation-induced plasticity. The overall mean spontaneous rate on the CT-intact versus the cut side was lower but not statistically significant ($t = 1.68$, df = 25.9, $P = 0.05$; $t = 2.10$, df = 5.4, $P = 0.086$; $t = 1.58$, df = 12.6, $P = 0.14$, respectively for nascinisor duct, foliate papillae, and soft palate). In addition, the median and maximal responses were greater on the cut side for all three receptive fields. Thus there appears to be a tendency for palatal and foliate responses to increase after denervation, even when differences in the number of responses is taken into consideration.

**TASTE RESPONSES EVOKED BY INDIVIDUAL TASTANTS.** The mixture analysis suggested denervation-induced changes in taste responsiveness, but because effects could vary according to quality, responses to individual tastants were also analyzed. We were particularly interested to determine whether NH4 Cl responses increased in magnitude following denervation. Instead of increasing, however, both NH4 Cl and NaCl responses decreased on the cut side, and only sucrose responses tended to increase (Fig. 6; ANOVA, $F = 7.60$, df = 4, $P < 0.001$ for interaction between tastants and side; $t = 3.08$, df = 64.1, $P < 0.005$; $t = 3.18$, df = 54.7, $P < 0.005$; $t = 1.79$, df = 42.6, $P = 0.08$, respectively, for NH4 Cl, NaCl and sucrose). Further, responses were greater on the cut side ($t = 2.73$, df = 58.5, $P < 0.01$; $t = 2.40$, df = 41.2, $P < 0.05$, respectively).

These differences may simply reflect the loss of CT input, or more interestingly, compensatory mechanisms triggered by denervation. For example, greater mean responses to palatal stimulation on the cut side were not unexpected, because there was an increased incidence of these responses on this side (see Receptive field organization). However, if comparisons are restricted only to suprathreshold responses, observed differences are more likely due to compensatory changes. These analyses have limited statistical power due to restricted sample sizes but show a consistent trend. Mean suprathreshold responses elicited by stimulating the nasoincisor duct, foliate papillae, and soft palate (bold numbers, 2nd line in insets, Fig. 5), were each larger on the cut side, although only for foliate stimulation was there a trend toward statistical significance ($t = 0.66$, df = 25.9, $P = 0.52$; $t = 2.10$, df = 5.4, $P = 0.086$; $t = 1.58$, df = 12.6, $P = 0.14$, respectively for nasoincisor duct, foliate, and soft palate).
it seems likely that these differential effects simply reflect the greater sensitivity of the CT to salts, and the greater superficial nerve to sucrose (Nejad 1986; Sollars and Hill 1998; Travers et al. 1986), along with the increased proportion of palatal neurons on the cut side. Because few individual receptor subpopulations were tested with single tastants, however, restricted analyses like those performed for the mixture were not possible. Another approach to directly compare nonanterior tongue responses was to remove all anterior tongue-responsive neurons from the data set and analyze whole mouth responses. This approach was less comprehensive because many neurons with convergence from the anterior tongue and other receptor subpopulations could not be used, but still resulted in a reasonable sample size. Using this strategy, there was no significant interaction between tastants and side, suggesting that the relative chemical responsiveness of residual taste responses was unaltered by denervation (ANOVA, $F = 0.76$, df = 4, $P = 0.56$).

In addition to comparing response magnitudes, correlation coefficients were calculated between stimulus pairs. This measure has been proposed to reflect the perceived similarity of two stimuli (Doetsch and Erickson 1970; Pfaffmann 1959; Smith et al. 1983). Because neonatally transected rats exhibit increased behavioral generalization between NaCl and NH₄Cl (Sollars and Bernstein 1996), we predicted an increase in the correlation between NaCl and NH₄Cl on the cut side. However, contrary to this prediction, correlations between responses to these stimuli were lower on the CT-cut side (Pearson correlation coefficients, $r = 0.728$ intact side, $r = 0.570$ cut side).

**Neuron types**

Finally, we examined chemosensitive response profiles using hierarchical cluster analysis (Fig. 7). A difference in che-
mosensitive groups could imply that afferent inputs were reordered after denervation. A scree analysis of the cluster tree suggested four groups of cells on the CT-intact side (Fig. 7, top panel), although two groups included most (36/41) neurons. Mean profiles are depicted in the left panel of Fig. 8. The largest group (n = 28) was the “NH” cluster. These cells nominally responded best to NH4Cl, but their mean response to NaCl was almost as great. These cells were heterogeneous, encompassing those that responded better to NaCl, better to NH4Cl, or similarly to both. The next-largest cluster (n = 8) included neurons that responded best to sucrose (“S” cluster), but this group also exhibited notable sideband responsiveness to both salts. The remaining neurons responded optimally to HCl or quinine, and these few “H” and “Q” cluster neurons had low response rates. The chemosensitive groups tended to have unique receptive fields, consistent with peripheral nerve sensitivities (Frank 1991; Nejad 1986; Sollars and Hill 1998; Travers et al. 1986). Most (79%) NH neurons responded to the anterior tongue, and these receptors usually comprised the optimal receptive field. S cluster neurons also received anterior tongue input, but less frequently (50%), and except for one S neuron, the foliate papillae or palate was the optimal receptive field. Only one H or Q cluster neuron had any input from the anterior tongue; instead their receptive fields included the foliate papillae or palate.

A cluster analysis suggested five instead of four chemosensitive types following CT transection (Fig. 7, bottom panel). However, except for the extra group, relative response profiles strongly resembled those on the intact side (Fig. 8). For each group the order of effectiveness of the five stimuli was virtually identical. Further, most cut-side cells (24/31) were also NH or S neurons. Despite these broad similarities, NH and S cluster neurons did appear more narrowly tuned on the cut side. For NH neurons, there was a 77% reduction in the sodium, but only a 35% decrement in the ammonium response and consequently, on average, these cells responded more specifically to NH4Cl. Indeed, in contrast to the intact side, each NH cell on the cut side responded best to NH4Cl. In addition, proportions of S versus NH cells varied between the two sides. There were equal numbers on the cut side but NH cells outnumbered S cells by a 3:1 ratio on the intact side.

Although the proportions and responsiveness of S and NH neurons exhibited differences, it seems doubtful that they indicate active plastic changes. Rather, the profiles on the cut

![FIG. 6. Mean (±SE) responses to whole mouth stimulation with individual tastants: 0.3 M sucrose (S), 0.3 M NaCl (N), 0.3 M NH4Cl (NH4), 0.01 M HCl (H), or 0.003 M quinine hydrochloride (Q). Mean responses to NaCl and NH4Cl were less on the cut side. Significant differences (independent t-tests; P < 0.05) are indicated by asterisks.](http://jn.physiology.org)
side appear to simply represent the chemosensitivity that remains after removing anterior tongue input. The middle panel of Fig. 8 depicts response profiles for cells on the intact side that did not receive anterior tongue input. Their numbers are too few for statistical analysis, but it is worth noting that they resembled corresponding groups on the cut side. There were equal numbers of nonanterior tongue S and NH neurons, and they tended to be more narrowly responsive. Finally, although infrequent, it was interesting that a novel cell type was delineated on the cut side. Four cells responded very specifically, albeit weakly, to NaCl. Another distinguishing feature of these cells was that three of four responded best to soft palate stimulation. Somewhat surprisingly, cells with a similar specificity for NaCl did not occur on the intact side. Although a subcluster from the NH group (n = 9) responded better to NaCl, except in one case, both salts evoked suprathreshold responses.

**DISCUSSION**

**Orotopic representation and receptive field organization**

This study was designed to determine whether neonatal CT nerve transection leads to changes in the orotopic representation or other changes in single-unit gustatory responses in NST. However, the orderly organization of AOC and POC taste responses was unaltered following unilateral CT nerve transection in young rats. We tested the hypothesis that POC taste-responsive neurons would shift rostrally on the CT-cut side. Instead, POC responses were found in symmetrical locations, caudal and medial to AOC responses.
orotopic organization on both sides was virtually identical to that reported in our previous studies (Dinkins and Travers 1998; Travers et al. 1986; Travers and Norgren 1995), making it unlikely that changes occurred but were masked because they occurred bilaterally. The lack of an orotopic reorganization suggests that large-scale functional changes that would be expected if anatomic changes (e.g., axonal sprouting or increased dendritic arborization) took place, do not appear to occur in the NST following CT transection in 10-day-old rat pups. Instead of receiving novel afferent inputs from other receptor subpopulations, the rostral pole of the NST (rostral 20%) was less responsive to taste stimulation on the CT-cut side. In the multiunit experiments 2 of 16 tracks on the CT-cut side were taste-responsive, compared with 12 of 17 on the intact side. Likewise, only 5 taste-responsive single units were isolated on the cut side versus 10 on the intact side.

Contrary to the present results, changes in topographic representation after peripheral denervation are common in other sensory systems. Median nerve transection results in altered responses in the primary somatosensory cortex in monkeys (Merzenich et al. 1983a; reviewed in Buonomano and Merzenich 1998; Merzenich et al. 1988). After denervation, neurons that previously responded to the denervated receptive field respond to an adjacent receptive field innervated by an intact nerve (e.g., the ulnar nerve). Studies over the last decade have shown that somatotopic reorganization in the somatosensory cortex of monkeys after peripheral deafferentation are reflected in similar changes at lower levels of the neuraxis, including the spinal cord and medulla (Florence and Kaas 1995; Garraghty and Kaas 1991a). These denervation-induced shifts in topographic organization are thought to reflect a functionally adaptive capacity for activity-induced plasticity (Jenkins et al. 1990). Changes result not only from reduced afferent drive, as occurs after denervation, but also from increases in sensory activity. A striking instance occurs in the somatosensory system of people who play string instruments; they exhibit larger areas of cortex dedicated to fingering digits than nonfingering digits (Elbert et al. 1995).

The lack of an orotopic reorganization in the present study could be taken to suggest that topography is less plastic in the gustatory system. Indeed, coding the location of a sapid stimulus is probably not a very important function of the taste system, and activity-dependent plasticity for taste bud subpopulations would be of limited use given that all are simultaneously stimulated during normal function. On the other hand, orotopic organization in the mammalian taste system is coarse, and we studied it at a relatively crude level, limiting the sensitivity of our assay to major shifts. In addition, we studied only a single time point after denervation. Although plasticity can occur just after denervation (Merzenich et al. 1983b; Xu and Wall 1997), denervation can also temporarily render the central representation silent (Merzenich et al. 1983b, 1984), and the most dramatic topographic shifts have been reported after lengthy periods (years) of denervation (e.g., Pons et al. 1991). In addition, central reorganization is often confined to a critical period (Wiesel and Hubel 1963). With regard to the current findings, it is relevant that decreases in CT terminal field volume after neonatal anterior tongue cautery occurred when this manipulation was performed at 2, but not 10 days of age (Lasiter and Kachele 1990). We chose the timing of denervation and testing in the present study to match conditions under which behavioral changes in salt responsiveness are apparent (Sollars and Bernstein 1996). Although we can rule out topographic shifts in the first-order relay as an explanation for this instance of behavioral plasticity, such changes could certainly occur with earlier or longer periods of denervation.

Increased convergence from multiple receptive fields would also indicate functional changes in NST. However, the degree of convergent input from intact nerves in the NST did not change after CT transection. A considerable amount of convergence normally occurs in NST between individual receptor subpopulations, especially between the anterior tongue and nasoincisor ducts (Dinkins and Travers 1998; Travers et al. 1986; Travers and Norgren 1991, 1995). Because convergence between the anterior tongue and other receptor subpopulations could not occur on the cut side, it is somewhat surprising that the degree of convergence remained the same. This is probably due to the greater number of palatal-responsive cells on the cut side, because these cells displayed frequent convergence between nasoincisor duct and soft palate receptor subpopulations. Therefore cells that responded to anterior tongue and other receptor subpopulations on the intact side were replaced with cells that responded to both palatal receptor subpopulations.

**Spontaneous firing rates**

Although neither changes in orotopy nor the organization of receptive fields were observed, spontaneous rates of NST cells were different between sides. Interestingly, we found an increase in mean spontaneous rate of nasoincisor duct-responsive cells after CT transection. However, in a previous study of the effects of CT anesthesia, we found the opposite effect for the same cell type; a trend toward a decrease in spontaneous rate (2.99 ± 1.18 spikes/s before anesthesia vs. 0.56 ± 0.20 spikes/s during anesthesia; t = 2.025, df = 9.5, P = 0.072, see Fig. 9) (Dinkins and Travers 1998). Because there was no significant difference in the spontaneous rate of nasoincisor duct-responsive cells on the intact side of this study and those

**FIG. 9.** Mean (±SE) spontaneous rates of nasoincisor duct-responsive (NID) cells from 2 studies. In contrast to an increased mean spontaneous rate after CT transection (current study), a trend for a decreased mean spontaneous rate was found during CT anesthesia (Dinkins and Travers 1998) (t = 2.55, df = 33.8, P < 0.05; t = 2.02, df = 9.5, P = 0.07, respectively; significant differences indicated by an asterisk).
from the anesthesia study (before CT anesthesia), we can rule out potential variables between studies (1.58 ± 0.48 spikes/s intact side vs. 2.99 ± 1.18 spikes/s CT anesthesia study; \( t = 1.11, df = 12, P = 0.29 \)). Although it is tempting to speculate that chronic denervation led to reactive changes in the CNS to account for the difference in the direction of spontaneous rate changes of CT-denervated animals, this could also be due to different peripheral reactions to the denervation methods used; i.e., anesthesia versus transection. Chorda tympani anesthesia may result in decreased spontaneous rate by decreasing peripheral input, but CT transection may trigger neuroma formation in the proximal nerve. In the somatosensory system, damaged peripheral nerves can form spontaneously active neuromas (Wall and Gutnick 1974). If this also occurred after CT transection, it would be expected to result in increased spontaneous activity in NST cells with CT input. Under denervated conditions, nasoincisor-duct responsive cells should be affected because anterior tongue and nasoincisor duct responses often converge. Unfortunately, the possibility of neuroma formation was not considered at the time of the experiment and hence cannot be ruled out. Similar to the somatosensory system, where spontaneous activity from neuromas has been implicated as a cause of phantom limb pain (Nystrom and Hagbarth 1981), if such neuromas do form after CT transection, they may be a cause for “dysgeusias,” i.e., unpleasant tastes of unknown origin (Bull 1965; Miller and Bartoshuk 1991), or “burning mouth syndrome” (Bartoshuk et al. 1996).

**Evoked firing rates**

Also contrary to our CT anesthesia study (Dinkins and Travers 1998), which provided no evidence that anesthesia caused mean taste responses to increase, the present results are suggestive that denervation resulted in increased responses arising from receptor subpopulations innervated by nerves other than the CT. Across all cells, taste mixture responses to nasoincisor duct and soft palate stimulation were greater on the cut side. Even when restricted to cells responsive to a particular receptive field, a trend for increased palatal and foliate responses persisted. In addition, the proportion of cells that responded to the soft palate was greater. Taken together, these results provide some evidence that GL- and greater superficial petrosal-mediated responses become more efficacious after CT denervation. This type of neural compensation could explain why human patients do not report deficits in taste after CT transection, even if such neuromas do form after CT transection, they may be a cause for “dysgeusias,” i.e., unpleasant tastes of unknown origin (Bull 1965; Miller and Bartoshuk 1991), or “burning mouth syndrome” (Bartoshuk et al. 1996).
though these cells were few in number and minimally responsive. It is interesting that the residual salt sensitivity we observed was attributable mainly to neurons with optimal receptive fields on the palate. This sensitivity is consistent with recent data (Sollars and Hill 1998) that show substantial sodium and ammonium responsiveness in the greater superficial petrosal nerve.

The current results also support previous neurophysiological data (Nejad 1986; Sollars and Hill 1998; see also Harada and Smith 1992), suggesting that the greater superficial petrosal nerve is more important than the CT in conveying information about “sweet” tastants in the rat. More neurons on the cut side responded to palatal stimulation, and sucrose was the only tastant that tended to elicit a larger response on that side. Differential contributions to sucrose responsiveness were also evident in the receptive field organization of S cluster neurons. Although a majority of intact side neurons responded best to anterior tongue stimulation, only a single S cluster neuron did. Instead, about one-half of the S neurons responded most vigorously to the palate and one-half to the foliate papillae. On the cut side, proportions of S cluster neurons increased in tandem with neurons that responded best to palatal stimulation. The current observations agree with our earlier results in NST, which found no sucrose-best neurons that only responded to the anterior tongue, but a substantial number with convergent input from the anterior tongue and nasoincisor duct (Travers et al. 1986). The present results further suggest that many S cluster neurons have receptive fields on the soft palate or foliate papillae.

**Neonatal CT transection: behavioral versus NST responses**

A striking increase in behavioral preference for NH₄Cl after CT transection in 10-day-old rats was demonstrated by Sollars and Bernstein (1996), yet the physiological changes that account for the behavior remain unknown. A simple hypothesis was that neurons in the rostral pole of NST that responded preferentially to stimulation of the anterior tongue with NaCl would be replaced by cells that responded broadly to NaCl and NH₄Cl applied to the posterior tongue. Corollaries of this hypothesis predicted increases in the magnitude or number of posterior tongue and NH₄Cl responses. This simple hypothesis is clearly untenable. As discussed above, instead of being more responsive to posterior tongue taste stimulation, the rostral pole of NST was less responsive to taste stimulation in general. Instead of being more responsive to NH₄Cl, the mean response to whole mouth NH₄Cl stimulation was reduced. Augmented responses to foliate stimulation suggest increased synaptic efficacy of the GL, but the proportion of neurons driven by this receptor subpopulation actually declined. Despite a thorough analysis, no other alterations in neural response that might explain the behavior emerged.

Although we did not find a correlate of increased NH₄Cl preference, we did note a change in NH₄Cl responsiveness that should have behavioral consequences. The Pearson correlation between NaCl and NH₄Cl decreased on the cut side. This decrease persisted when restricting the analysis to cells without CT input, suggesting that it may reflect plasticity rather than merely a difference in anterior tongue versus nonanterior tongue responsive cells. However, a relatively small number of cells without CT input (n = 11), were available for this analysis, making this interpretation tentative. Further, the change was opposite to that predicted from behavioral observations. In addition to the preference shift, rats with neonatal CT transections generalize more between NaCl and NH₄Cl (Sollars and Bernstein 1996), a change that should be accompanied by an increase in across-neuron correlations. This discrepancy between the neural and behavioral results is difficult to explain.

The lack of plastic changes in NST responses consistent with the behavioral changes induced by neonatal CT transections (Sollars and Bernstein 1996) implies that neural correlates of the altered behavior reside elsewhere, or that our experiment failed to reveal them. It should be noted that the present study did use a different rat strain (Long-Evans) than the one (Wistar) used in the behavioral experiment (Sollars and Bernstein 1996), and it is possible although it seems unlikely that this disparity would have obscured such changes. Another difference between the behavioral experiment (Sollars and Bernstein 1996) and the present investigation was that the former study used bilateral cuts but that we sectioned the CT unilaterally and compared the two sides of the brain. It is possible that the neural plasticity requires bilateral cuts. However, this also seems unlikely. In most models of deafferentation-induced plasticity, unilateral transection produces profound neural changes (e.g., Jones and Pons 1998; Merzenich et al. 1983a; Pons et al. 1991; Wiesel and Hubel 1963). Thus it seems more likely that plasticity at higher levels of the gustatory neuraxis underlie the behavioral change. This would be consistent with other sensory systems. Although plastic changes certainly occur at subcortical levels (Florence and Kaas 1995; Garraghty and Kaas 1991a), alterations are cumulative and are larger at higher levels of the neuraxis (Florence and Kaas 1995; Florence et al. 1998; Garraghty and Kaas 1991b). The competence of brain stem circuits for making basic gustatory discriminations (Grill and Norgren 1978) suggests the parabrachial nucleus as a possibility. However, the complexity of taste preference behavior also favors several forebrain regions.

We thank Dr. Ilene Bernstein and M. Roitman for initial discussions and for demonstrating the CT transection procedure. We thank Drs. Keith Alley, J. P. Baird, Scott Herness, and Joseph Travers for helpful comments on previous versions of the manuscript. We greatly appreciate the excellent technical assistance provided by E. Hauswirth, Dr. Hecheng Hu, and K. Urbanek. This work was supported by National Institute of Dental Research Grant DE-00357 (Dentist-Scientist Award) to M. Dinkins and National Institute on Deafness and Other Communication Disorders Grant DC-00416 to S. Travers. Received 18 March 1999; accepted in final form 2 July 1999.

**REFERENCES**


