Recovery of Functional Response in the Nucleus of the Solitary Tract After Peripheral Gustatory Nerve Crush and Regeneration

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Barry, Michael A. Recovery of functional response in the nucleus of the solitary tract after peripheral gustatory nerve crush and regeneration. J. Neurophysiol. 82: 237–247, 1999. Single-unit recording and transganglionic tracing techniques were used to assess the properties of, and inputs to, neurons within the rostral nucleus of the solitary tract (NST) after peripheral gustatory nerve injury and regeneration in adult hamsters (Mesocricetus auratus). Tastant-evoked responses were recorded from 43 neurons in animals in which the ipsilateral chorda tympani (CT) nerve was crushed 8 wk earlier (experimental animals) and from 46 neurons in unlesioned control animals. The 89 neurons were separated into three functional clusters named according to the best stimulus for neurons in the cluster: S, sucrose; N, sodium acetate; and H, HCl or KCl. Stimulus-evoked spike rates across all stimuli were 35.4 ± 4.4% lower in the experimental hamsters. The largest difference in evoked spike rates occurred for neurons in the H cluster, in which the response to KCl also was delayed relative to normal responses. The response of S-cluster units to sucrose and saccharin was also lower in the experimental animals. The mean response rate and the time course of response of neurons in the N cluster did not differ between the two groups. For each cluster, the spontaneous rates and mean response profiles across eight stimuli were very similar in the experimental and control animals, and the breadth of tuning hardly differed. In both groups, Na⁺ responses in the N cluster were amiloride sensitive, and responses to the water rinse after stimulation with HCl were common in the S cluster. At 8–20 wk after nerve crush, biotinylated dextran tracing of the CT nerve revealed that the regenerated CT fibers did not sprout outside the normal terminal zone in the NST, but the density of the central terminal fibers was 36.9 ± 6.35% lower than normal. After CT nerve crush and regeneration, the overall reduction in taste-evoked spike rates in NST neurons is likely a consequence of this change in terminal fibers; this in turn likely results from the known reduction in CT fibers regenerating past the crush site. In the face of this reduction, the normal taste-evoked spike rate in N-cluster neurons requires explanation. The observed recovery of normal specificity could be mediated by a restoration of specific connections by primary afferent fibers peripherally and centrally or by central compensatory mechanisms.

INTRODUCTION

After peripheral nerve damage in mammals, gustatory nerves regenerate, reinervate taste buds, exhibit physiological responses to gustatory stimuli, and can mediate taste-guided behaviors (Cheal and Oakley 1977; Cheal et al. 1977; Hill and Phillips 1994; Hillerup et al. 1994; Ninomiya 1998; Robinson 1989; St. John et al. 1995; Whitehead et al. 1995; Zuniga et al. 1994, 1997). In the case of golden hamsters, a regenerated chorda tympani (CT) nerve supports a normal population and size of fungiform taste buds after complete crush injury, despite the loss of ~67% of myelinated fibers (Cain et al. 1996). Whole nerve recordings from the regenerated nerve reveal a normal range of responsiveness to chemical stimulation of the anterior tongue but more variable responses to sucrose and a greater than normal frequency of responses to water rinses after gustatory stimuli (Cain et al. 1996). The regenerated chorda tympani nerves can mediate a conditioned taste aversion specific for NaCl (Barry et al. 1993b).

The central mechanisms by which this behavioral recovery is achieved have not been addressed, and the central consequences of a reduced number of afferent fibers are not clear. The recovery of the pattern of sodium taste-induced staining for c-fos in the hamster parabrachial nucleus after CT nerve crush and regeneration (Barry and Larson 1993), suggests that normal circuitry may be retained or reestablished. However, after gustatory nerve damage, there are changes in the first central gustatory relay, the nucleus of the solitary tract (NST), including transganglionic degeneration (Whitehead et al. 1995), increased glial fibrillary acidic protein (GFAP)–like immunoreactivity (Smith et al. 1993), and reduced staining for acetylcholinesterase (Barry and Frank 1992). Long-term changes include incomplete recovery of acetylcholinesterase staining (Barry et al. 1991) and persistence of degeneration products for months (Whitehead et al. 1995). The presence of these products could reflect ongoing degeneration such as that seen in the auditory system (Morest et al. 1998). To better understand the basis for recovery of this system after nerve damage, it is important to explore whether there is compensation in the NST for the reduction in regenerated primary afferent fibers and if NST neurons exhibit normal specificit.

A recovery of normal specificity would be expected if regenerating CT afferent functional types (Frank et al. 1988) quickly establish connectivity with their appropriate peripheral and central targets and central networks remain intact. The restoration of normal peripheral connectivity is probable because during the normal course of receptor cell turnover (Beidler and Smallman 1965; Farbman 1980), the primary afferent fibers must make appropriate connections with replacement receptor cells (Frank et al. 1988). However, at early stages of recovery, there could be a loss of specificity in NST neurons due to incorrect central connectivity of primary afferent fibers and possible degradation of central circuitry.

This study examines the response to tastants of NST neurons 8 wk after complete crushing of the CT nerve in hamsters. The
8-wk stage is the earliest post-crush time when consistent CT responses have been recorded in hamsters (Cain et al. 1996). The results revealed a recovery of normal functional types and specificity. Across all neurons, taste-evoked spike rates were lower than normal, but there was an unexpected differential recovery in certain functional types.

METHODS

Subjects

A total of 10 control (normal) and 14 experimental nerve crush (crush) adult (120–170 g) male golden Syrian Hamsters (Mesocricetus auratus) were used for the electrophysiological study. An additional seven male hamsters were used for anatomic studies. All procedures were in accordance with National Institutes of Health guidelines and approved by the committee for laboratory animal care at the University of Connecticut Health Center.

Surgery

For exposure of the CT nerve, the hamsters were anesthetized with pentobarbital sodium (90 mg/kg ip; Nembutal, Abbott Laboratories) and placed in a nontraumatic head holder. The CT was exposed by opening a small hole in the dorsal margin of the tympanic membrane just caudal to the malleus. The nerve was crushed at a single point with No. 5 forceps until only a thin strand of nerve sheath remained (5–15 crushes). Buprenorphine (Buprenex, 0.5 mg/kg sc) was administered as a prophylactic analgesic every 12 h for 8 h after surgery. Control (normal) animals received no manipulations. The experimental animals were all allowed to survive 8 wk before electrophysiological experiments.

For physiological studies, all animals were anesthetized with urethan (425 mg/ml water, Sigma) at an initial dose of 1.59 g/kg with supplemental doses of 0.17 mg/kg as necessary. After a tracheotomy, the hamster was placed in a nontraumatic head holder with the head angled downward at a 19° angle from horizontal (McPheeters et al. 1990). The brain stem was exposed by removing the occipital bone and by aspirating most of the cerebellum.

Location of the taste-responsive area

The taste-responsive area of the NST initially was located 2.5 mm anterior and 1.5 mm lateral to the observable convergence of the gracile nuclei at the caudal end of the fourth ventricle (McPheeters et al. 1990). Multiunit activity was recorded with glass microelectrodes (broken to 40 μm; Sutter BV-10) to an impedance of 16–20 MΩ. Extracellular single-unit activity was recorded with a Dagan 2400 amplifier. Gustatory units were identified based on their responsiveness to the search solution; all units were accepted that appeared to show a consistent increase in spike rate to multiple applications of the search solution to the anterior tongue. Waveforms were amplitude discriminated on-line and saved using the Experimenter’s Workbench program (Datawave).

Stimuli

Stimuli were dissolved in distilled water, delivered at room temperature, ~23°C, and presented in two sets: the first consisted of 0.03 and 0.1 M sodium acetate (NaAc; N1 and N2), 0.1 and 0.3 M sucrose (S1 and S2), 0.1 and 0.3 M KCl (K1 and K2), 0.01 M HCl (H), 0.003 M saccharin (saccharin), water, or a search solution consisting of (in M) 0.1 NaAc, 0.3 KCl, 0.3 sucrose, and 0.003 M saccharin. The second set repeated all of the above stimuli except the low concentrations of NaAc, sucrose, and KCl and also included the sodium channel blocker, amiloride (10 μM) alone or added to 0.1 M NaAc, 0.3 M sucrose, 0.01 M HCl, and 0.3 M KCl.

The tongue was held out of the mouth with the aid of a small weight clipped to the contralateral side of the tongue. A tube was used to direct the stimulus flow to the anterior half of the tongue; thus the most caudal fungiform, as well as foliate and vallate taste buds were not likely to be stimulated. For units where the evoked response seemed slow to develop or lasted beyond the end of the stimulus, the stimulus tube was moved to only the very tip of the tongue, and the pattern of response always remained. The stimulus control system was described previously (McPheeters et al. 1990). The flow rate was 26.4 ml/min.

Stimulus timing was controlled by a computer with Experimenter’s Workbench software (Datawave). The timing sequence for stimulus presentation in set 1 was a 15-s water rinse (pre rinse), followed by 10 s of a taste stimulus, a 20-s water rinse, and a 45-s period without stimulus flow. Thus the stimuli were separated by 1.5-min intervals. For stimuli set 2, the protocol was largely the same except that a 1-s flow of 10 μM amiloride alone was applied before and after stimuli that included amiloride. In addition, another paradigm to test the effects of amiloride on the response to NaAc was used (Hettinger and Frank 1990) that was useful for units with a high net response rate. A 6-s stimulus of 0.1 M NaAc was followed by an 8-s period of 0.1 M NaAc mixed with 10 μM amiloride and another 6-s period with NaAc alone.

For each unit, the response to tactile stimulation of the tongue was crudely assessed. A paint brush (size 1) was used to stroke or poke the tongue. An increase in activity over a baseline was assessed subjectively.

Histological reconstruction

In three crush and two control animals a glass micropipette (broken to ~3 μm) containing 2% pontamine sky blue (BDH/Gurr) in 0.5 M Na acetate (pH 7.7) was lowered at the same coordinates where single units responded to gustatory stimulation had been found. Multiunit activity evoked by the search solution was used to verify electrode placement. Then a negative voltage of 15 V (~4 μA, Dagan 2400) was applied for 15 min. After ~1 h, the still deeply anesthetized hamsters were perfused intracardially with heparinized phosphate buffered saline followed by 10% formalin in 0.1 M phosphate buffer (pH 7.4). After infiltration with 30% sucrose in phosphate buffer, the medulla was serially frozen sectioned in the transverse plane at 40 μm.

Data analysis

Units were only included if the first set of stimuli was completed. Part or all of set 2 responses were obtained for 30 of 43 crush units and 28 of 46 normal units.

Spike Sort (Common Processing software, Datawave) was used to verify that the stored waveforms represented single-unit spike activity and to cut out any artifactual waveforms. Except for two recordings, in each of which two units were isolated, all the recordings represented single units. All units appeared, based on the shapes of their action potentials, to originate from neurons. Unit recordings from fibers are unlikely due to their small (~1 μm diam) size. The spon-
taneous rate for each stimulus was defined as the mean spike rate during the 10-s period immediately preceding the stimulus. Net spike rates were calculated by subtracting the spontaneous rate from evoked spike rates. Stimulus response onset was defined as the time the stimulus reached the tongue or 1 s later as determined by which time resulted in the largest net spike rate over the following 5 s of the stimulus. The exception was a single crush neuron where the response was unusually delayed; the response onset was instead set at 5 s after the stimulus.

The 5-s net response rates to the eight stimuli in the first stimulus set were analyzed further to reveal neuron types. Pearson’s product correlations (Statistica) were computed between all 89 neurons; normal and crush units were combined to facilitate comparisons. These correlations were used as the basis of a hierarchical cluster analyses using the weighted-average linking method (Statistica) as used in other studies on NST neurons (Giza and Scott 1991; Jacobs et al. 1988). Clusters and subclusters were identified based on the criteria of a Pearson’s $r$ of $\leq 0.6$ and at least five neurons.

A MANOVA (Statistica), with condition (normal vs. crush) and cluster as independent variables and the net 5-s response to each of the stimuli as the dependent variable, was used to analyze the differences between the normal and crush neurons. Similar MANOVAs or ANOVAs (SPSS or Statistica) were used to examine other measures including spontaneous rate and the breadth of tuning. To examine the effects of the inhibitor amiloride, the analyses were similar but the responses with and without amiloride were treated as repeated measures. Except as noted, $F$ tests for planned comparisons were used only if warranted by the significance of the overall analyses. For MANOVAs, the multivariate $F$ (Rao’s $R$) value is provided.

A measure of the specificity of each unit was computed using the breadth of tuning metric (Smith and Travers 1979)

$$H = -k \sum_{i=1}^{n} P_i \log P_i,$$

where $P$ is the proportion of a given net response relative to the total of net responses across four stimuli (N2, S2, K2, H). The constant $k = 1.661$ for four stimuli. To prevent calculation problems for net spike rates of less than zero, the response rates for that unit were normalized so that the lowest response rate equaled 0 spikes/s. Values of $H$ close to zero indicate sensitivity to a single stimulus (narrow tuning); values close to 1.0 indicate sensitivity to all four stimuli (broad tuning).

When examining individual neurons, the criterion for an evoked response was an increase in spike rate from spontaneous of 1.65 SD ($P < 0.05$) for a 5-s period. For the determination of water responses by individual neurons, the spike rates during the 5-s before and after each water pre rinse were compared with Student’s $t$-tests. Means ± SE are used throughout.

Verification of regeneration

In three crush cases, we visually examined the CT nerve in the middle ear to confirm regeneration, and in our previous work, 100% of crushed CT nerves regenerate (Cain et al. 1996). There is no evidence for the sprouting of gustatory nerves to innervate neighboring denervated peripheral fields (Kinnman and Aldskogius 1988; Zalewski 1969); for further references, see Barry and Frank 1992), and stimuli were directed to the anterior tongue in the present study. Thus evoked responses must have been mediated by the regenerated CT nerve.

Transganglionic anterograde tracing of CT central projections

Transganglionic anterograde tracing was used to ascertain if after peripheral regeneration there was sprouting of primary afferent fibers beyond the normal NST terminal field and whether there was a decrease in the central density of regenerated CT fibers and terminals corresponding to the reduction in peripheral fibers (Cain et al. 1996). As part of another ongoing study of the fine structural morphology of central terminals, hamsters were anesthetized and the CT nerve was crushed unilaterally as described in the preceding text, except that the crushes were made just rostral to the malleus. At survival times of 8 ($n = 3$), 16 ($n = 2$), and 20 ($n = 2$) weeks, the animals were anesthetized, and both CT nerves were exposed and severed just caudal to the malleus (2 mm central to the crush site) in the middle ear. About 0.5 mg of lyophilized biotinylated-dextran crystals (10 kDa, Molecular Probes) were applied to the proximal cut end of each nerve. After a further survival time of 2 days, the animals were anesthetized and perfused with heparinized phosphate buffered saline followed by 3% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer.

Forty-micrometer vibratome sections of the brain were incubated overnight at 4°C in ABC solution (elite kit, Vector). The sections were stained with diaminobenzidine, osmicated for 1 h in 0.5% OsO$_4$, dehydrated, infiltrated, and flat-embedded in a Mollenhauer mixture of araldite and epoxy (Polysciences). For this study, light microscopic images ($\times 10$ objective) were captured with a video frame grabber for analysis with image processing software (IPLab Spectrum, Signal Analytics). Optical densities were measured within a 31.5 $\times$ 31.5 $\mu$m ($30 \times 30$ pixels) square. On both the intact and crushed nerve sides of each section, a single measurement was taken from the most densely stained area within the CT terminal zone, and a control (background) measurement was made in the ventral NST. The density of the diaminobenzidine stained area was expressed as a ratio over background. The differences between the intact and crushed sides were tabulated, and a single mean difference was calculated for each animal. For measurements of the area of the terminal field, a line was drawn around the stained terminal fibers in the NST. The results from the animals ($n = 7$) from the different survival periods (8–20 wk) were examined together. Statistical differences between the intact and crush sides were evaluated with Wilcoxin matched-pair tests. The results do not address the fate and possible central projections of geniculate ganglion neurons whose peripheral axons do not regenerate because the site of dextran application in the middle ear was very close to the crush site.

RESULTS

General and cluster analysis

Neuronal responses to taste stimulation of the anterior tongue with all eight stimuli were obtained readily from the rostral NST in both the normal and crush hamsters. The response profile across tested tastants for crush neurons was similar to normal (Fig. 1). There was however a decrease in the evoked response rate in the crush group (see following text).

The cluster analysis resulted in three main clusters; normal and crush neurons were found in all clusters (Fig. 2). The three clusters corresponded to neurons that responded best to sucrose (cluster S), NaAc (N), and KCl or HCl (H) (Fig. 3). Seven subclusters also were identified (in italics in Fig. 2) The subclusters are named based on their best and second best stimulus. Most statistical analyses were applied only to the three large clusters due to sample size limitations. The significance of the interactions in responses among the three clusters was supported by the results of a MANOVA analysis; cluster had a significant overall effect on the responses to eight taste stimuli (N1, N2, S1, S2, Sac, K1, K2, and H); $F(16,152) = 16.30, P < 0.001$. Also there was a significant difference when the response of the best stimulus for each cluster was contrasted with
the response to that stimulus in the other three clusters. For example for neurons in the N cluster, the response to N2 was significantly greater than that of neurons in the other two clusters; \(F(1, 83) = 23.13, P < 0.001\).

### Response rate

There was a mean decrease of 35.4 ± 4.4% in the net response rate to all stimuli in the crush neurons relative to normal; \(F(8, 76) = 2.0, P < 0.05\) (1-tailed) (Fig. 1). This effect was significant for the N2, K1, K2, and H individual stimuli. However, not all clusters showed similar differences between crush and normal neurons. The largest effects on spike rate were seen in responses to all salts in the H cluster (Fig. 3), and apparently in the Nh subcluster (Fig. 4, too few neurons for statistical analysis). In cluster S, there was a significant but relatively small decrease in spike rate in response to sucrose (S1 and S2). Most striking was the lack of a difference between conditions in the response to NaAc (N1 and N2) or other stimuli in the N cluster. This recovery of the normal response of these neurons to NaAc was in sharp contrast to the significant reduction in the response to NaAc in the H cluster neurons (Fig. 3).

### Spontaneous rate and breadth of tuning

The mean spontaneous rate for all neurons was <1 spike/s. Mean spontaneous rate did not differ significantly among the three clusters, \(F(1, 83) = 0.981\), NS, and was not affected by condition (Table 1). The specificity of neurons (measured by breadth of tuning) did not differ between the crush and normal neurons; there was an effect of cluster on breadth of tuning \([F(2, 83) = 4.64, P < 0.05]\) but not of condition; \(F(1, 83) = 0.018\), NS. An interaction between the cluster and condition effects was suggested by \(F(2, 83) = 2.67, P = 0.075\). An examination of the individual clusters revealed an effect in the N cluster only; there was a slightly greater breadth of tuning in the crush group; \(F(1, 83) = 3.16, P < 0.05\) (1-tailed).

### Response profiles and specificity

The patterns of response to stimuli for each of the three clusters and seven subclusters were similar in the crush and normal neurons (Figs. 3 and 4). Most (60/89) neurons were in three subclusters (K, N, and S) characterized by relatively high specificity. The mean breadth of tuning for neurons in these clusters was 0.57 ± 0.027 compared with 0.78 ± 0.025 for the other four subclusters. The effects of nerve crush on stimulus-evoked spike rates appeared to be independent of the breadth of tuning of the subclusters. In the H cluster, mean response rates for neurons in specific (e.g., K) and less specific (Hk) subclusters were both less than normal in the crush group (Fig. 4).

### Time course of response

The time course of responses to the best stimuli in the normal and crush neurons were virtually identical for the N cluster and very similar for the S cluster (Fig. 5). Thus differences between the evoked spike rates of normal and crush
neurons persisted over time in these clusters. For example, the response to sucrose (S1 and S2) of crush neurons in the S cluster was reduced both in the initial 5 s (Figs. 3 and 5) and the last 5 s of the stimulus; for S2, F(1,81) = 6.6, P < 0.05.

Nerve crush affected the temporal course of the response to KCl in the H cluster. In an ANOVA with experimental group as the independent variable and the initial and last 5-s evoked responses to KCl as a repeated measure variable, there was an interaction between the two variables; F(1,21) = 9.14, P < 0.05. As opposed to the initial 5 s of the stimulus (Fig. 3), during the last 5 s of the stimulus, the response to K2 was not affected by condition in the H cluster; F(1,83) = 0.20, NS (Fig. 5). Thus for K2 and K1 (data not shown), there were greater effects of nerve crush for the more phasic portion of the response. The responses to HCl under both conditions and to KCl in the crush neurons were characterized by the lack of a phasic response. However, for HCl, the temporal pattern appeared similar in the normal and crush groups (due to the limited number of neurons in the H cluster that showed strong responses to HCl, a separate ANOVA was not possible).

Effects of amiloride

Exposure of the anterior tongue to amiloride resulted in decreases in the response to NaAc across all neurons; F(1,51) = 12.42, P < 0.001. The response to amiloride did not differ between the normal and crush groups. There was a significant interaction between the cluster and amiloride variables; F(2,51) = 9.93, P < 0.001. Amiloride inhibited the response to NaAc only in the N cluster [F(1,51) = 36.29, P < 0.001]; mean net spike rates (during first 5 s)
decreased from 6.41 ± 1.77 to 2.7 ± 0.691 spikes/s in the normal group and from 6.22 ± 2.23 to 2.55 ± 1.03 in the crush group. In the second amiloride paradigm, where amiloride was introduced after the initial response to NaAc, most N cluster neurons showed a decrease in activity, but only a few neurons in both groups showed a recovery of the response to NaAc after removal of the amiloride (Fig. 6). ANOVA analyses revealed no overall (or interaction) effects of amiloride on responses to S2, K2, or H stimuli. The inhibitory effect of amiloride on the response to HCl in N cluster neurons was explored because of reports of limited amiloride sensitivity of the HCl response in hamsters (Hettinger and Frank 1990). There was a trend \( F(1,42) = 3.77, P = 0.059 \) for such an effect in the N cluster (experimental groups combined). Amiloride, when presented alone as a taste stimulus, resulted in a decrease in spike rate relative to spontaneous rate (from 0.67 to 0.24 spikes/s) in the N cluster \( F(1,83) = 6.61, P < 0.05 \) but had no effect in the other clusters.

**Water-rinse response**

Many crush and normal neurons showed an increase in activity associated with the water rinse after taste stimuli, particularly HCl (11/46 normal and 7/43 crush neurons). This effect of HCl was most common for neurons in the S cluster (11/20 normal and 5/13

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**TABLE 1. Spontaneous rates and breadth of tuning for NST units**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Spontaneous Rate*</th>
<th>Breadth of Tuning (H)†</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Crush</td>
<td>Normal</td>
</tr>
<tr>
<td>H</td>
<td>0.96 ± 0.21</td>
<td>0.68 ± 0.21</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>N</td>
<td>0.53 ± 0.16</td>
<td>0.91 ± 0.20</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>S</td>
<td>0.64 ± 0.18</td>
<td>0.48 ± 0.21</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>All units</td>
<td>0.67 ± 0.10</td>
<td>0.71 ± 0.11</td>
<td>0.63 ± 0.03</td>
</tr>
</tbody>
</table>

* Values are in spikes per second expressed as means ± SE. † Values are expressed as means ± SE (see METHODS).

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**FIG. 5.** Mean activity for neurons in 3 main clusters as a function of time. Stimuli were applied at time 0 for 10 s. Water, at the same flow rate, was applied for the 15 s before and 20 s after each stimulus as shown (bottom left).
crush neurons). The response was seen in neurons where HCl was also an excitatory stimulus (neuron Cru16 in Fig. 7), and where it was not (Cru12 in Fig. 8). In many neurons, the response to the taste stimulus appeared to persist >2 s after the end of the stimulus, and this was apparent in the mean responses (Fig. 5). This persisting response often made it difficult to determine if the activity during the water rinse was a result of a persisting response or a rinse response (neurons Cru16 in Fig. 7, Cru30 in Fig. 8). Comparable numbers of normal and crush neurons showed apparently persisting responses to stimuli other than HCl (23/46 normal and 20/43 crush neurons).

In the crush group, two S cluster neurons and one NS subcluster (Cru19 in Fig. 8) neuron showed rinse responses after sodium stimuli, and 1 N cluster neuron responded to the rinse after sucrose stimuli (C30 in Fig. 8). No normal neurons showed a clear rinse response after stimuli other than HCl (or weakly after KCl in the same neurons).

**Tactile response**

Responses to stroking or gently poking the tongue rarely were observed. Of 43 tested normal neurons, in response to touch, 1 responded consistently with a large increase in spike rate over spontaneous and 4 neurons responded relatively weakly. Only 3 of the 39 tested crush neurons responded to touch (all strongly). In a MANOVA analysis, there were no differences in spike rates between the 5-s periods before and after water flowed across the tongue. A more detailed analysis revealed that a few individual neurons in both groups responded significantly, with small (≤2 spikes/s) increases or decreases in activity in response to water, but these were not the same neurons that responded to touch.

**Location of recording sites**

All five (3 crush, 2 normal) dye injections were localized within the NST and within the normal CT terminal zone, which lies medial to the solitary tract in the dorsal part of the rostral central subdivision and rostral lateral subdivisions of Whitehead (Barry et al. 1993; Whitehead 1986, 1988). All the injections were ≤150 μm caudal to the rostral tip of the NST. In all but 1 of the 24 animals, the caudal extent of single-unit recordings was ≤250 μm from the rostral-most multiunit taste-responsive site (presumed rostral tip of NST). The mediolateral ranges of single-unit electrode penetrations was ≤100 μm. Thus all the single-unit recordings were probably within the region where the densest part of the CT terminal field is located (Barry et al. 1993a).
Central CT projections

The use of biotinylated dextran for transganglionic tracing resulted in intense labeling along with good morphology of the labeled fibers and terminal swellings. Both the intact and previously crushed nerves projected to the same region of the rostral NST within the rostral central and rostral lateral subdivisions (Fig. 9). The staining was affected by the condition. There was a mean reduction of $36.9 \pm 6.35\%$ in staining density (includes fibers and terminals) in the NST on the previously (8–20 wk) crushed side relative to the intact side; $Z(6) = 2.366, P < 0.05$. There was also a $23.03 \pm 4.41\%$ reduction in the area (measured in transverse sections) of the labeled terminal field on the crush relative to the intact side of the NST, $Z(6) = 2.366, P < 0.05$. The differences in staining density and area between the two treatments did not vary with position in the rostrocaudal axis of the NST. The transganglionic anterograde tracing results, together with the marking of recording sites, suggest that the single-unit recordings were made from the same population of central neurons in both the normal and crush animals.

DISCUSSION

Effects of nerve crush and regeneration

Overall rates of response to taste stimuli were lower than normal after CT crush and regeneration. This result is consistent with the reduced number (Cain et al. 1996), terminal density, and terminal area of regenerated afferent fibers and suggests an overall lack of central compensation for the reduction in afferent inputs. However, in the N cluster there was no difference between groups in mean response rates, time course of response, or amiloride sensitivity after CT crush and regeneration. Behavioral responses specific for sodium appear to be very dependent on the CT nerve in hamsters (Barry et al. 1996; Yamamoto et al. 1988) and recover after CT regeneration (Barry et al. 1993b). Thus there may be differential recovery of an important sensitivity of the CT in hamsters. Peripheral single-fiber recording studies are needed to see if the recovery of N-cluster neurons is due to peripheral or central compensatory processes.

After regeneration, the recovery of responsiveness to all tested stimuli suggests that there is a recovery of the normal complexity of receptor cell responses, including second-messenger pathways. For example, sucrose and saccharin were both effective stimuli and are thought to trigger different second-messenger pathways and may act at distinct receptors (Lindemann 1996).

Remarkably the overall specificity of responses was unchanged as measured by the breadth of tuning metric. Also the normal and crush group neurons appeared to be part of a single population in terms of their response profiles to taste stimuli overall and within each of the three clusters. Single-unit studies on regenerated gerbil CT fibers also found some very specific units (Cheal et al. 1977). Thus it is probable that regenerating primary afferent fibers make contact with or induce the differentiation of specific taste receptor cell types in the periphery. This specific contact or induction may be based on a specific property associated with individual primary afferent fibers. A cross-regeneration study in mice revealed that units in the CT and glossopharyngeal nerves retained their characteristic pattern of responsiveness to NaCl and amiloride despite innervating a different population of taste buds (Ninomiya 1998).

The normal specificity of NST neurons in the crush group could result from the reestablishment or retention of specific connections between primary afferents and NST neurons. A restoration of normal connectivity is implied by a study that showed a recovery of the normal pattern of cells with elevated c-fos activity induced by NaCl taste in the parabrachial nucleus (the gustatory relay to which the NST projects) (Barry and Larson 1993). However, central com-
The responses to KCl and HCl were most affected by CT crush in terms of both response rate and, for KCl, time course. Subtle changes in the time course of response of NST neurons may be important for coding changes in behavioral state such as conditioned preferences (Giza et al. 1997). Differential effects on responsiveness to HCl and KCl were not apparent from whole nerve recordings of the regenerated CT nerve (Cain et al. 1996) but were only revealed in the NST after a separation of neuronal types. Thus CT single-fiber studies are needed to determine if the effects on KCl taste are due to peripheral or central changes. Intact CT nerves are important for normal and learned responses to KCl in hamsters and rats (Barry et al. 1996; St. John et al. 1997). Thus the lack of recovery of normal KCl responses in the NST may be behaviorally important.

The finding of highly variable responses to sucrose by the regenerated CT nerve (Cain et al. 1996) was not seen in the NST. However, this variability was between animals (and more extensive at 4–5 than 8 wk survival periods) and thus probably would not have been revealed by the limited sampling possible in this single unit study.

**Sprouting**

Regenerating CT fibers did not sprout beyond their normal terminal zone in the NST; rather there was a slight decrease in the size of the terminal zone (measured in transverse sections). However, there could be sprouting of the regenerated afferent fibers within their normal terminal zone.

**Comparison with normal CT and other NST data sets**

The neuron types found in this study clearly relate to and, in many neurons, resemble the response of the three classes of primary afferent fibers found in the male golden hamster CT: S fibers (respond best to sucrose), N fibers (sodium/lithium salts), and H fibers (HCl, KCl, NaCl, and other salts) (Frank 1973; Frank et al. 1988). In the present study, sucrose, NaAc, and KCl were chosen because they enable the discrimination of the three peripheral classes of fibers. NaAc is an equally good stimulus as NaCl for receptor cells innervated by N fibers but is a relatively weaker stimulus for H fibers (Rehnberg et al. 1993).

In general, the types and amiloride sensitivity of central neurons observed in this study were similar to those seen in other studies of the hamster NST (McPheeters et al. 1990; Smith et al. 1983, 1996; Travers and Smith 1979). An exception was the response of K- and Sk-subcluster neurons to KCl but not to HCl. This separation of sensitivities is not generally seen in the periphery or centrally (Frank et al. 1988; McPheeters et al. 1990; Smith et al. 1996) but can be explained partially by differences in the stimuli. Most other studies used 0.1 M KCl, which was not a very potent taste stimulus for most of the K-subcluster neurons. Also NaCl is a better stimulus than NaAc for peripheral H fibers, thus K-subcluster neurons may have been interpreted as NaCl-best in other studies where only NaCl was used. The relative insensitivity of these neurons to HCl suggests that there may be a subset of peripheral H fibers responsive to KCl but not HCl.

Responses to stimuli often persisted well into the rinse period. Such persisting responses have not been emphasized in other reports on NST physiology. The persisting responses were not due to the stimulation of nonfungiform taste buds because whenever such responses were observed, the stimulus flow was directed to just the tongue tip to verify the pattern of response. The frequency of persisting responses did not vary between the two experimental groups.

**Water-rinse responses**

The strength of response to the application of water to the anterior tongue is species dependent, and normal hamster CT fibers respond rather weakly (Bartoshuk 1965). However, responses to the water rinses after taste stimuli in CT whole nerve recordings occur more frequently after CT crush and regeneration (Cain et al. 1996). A rinse response after acid stimuli was very common in both normal and crush NST neurons in the S cluster, although it was rare in the CT nerve after a lower concentration (0.003 M) of HCl (Cain et al. 1996). Rinse responses after stimuli other than HCl were rare in the NST. Clear examples were found in only a few NST neurons in the crush group and not in the control group. This result is consistent with the peripheral findings, but the dataset.
is too small to conclude that normal neurons never show such behavior.

Comparison with other sensory systems

The gustatory system is most similar in its peripheral organization to the vestibular/auditory and somatosensory systems in that all consist of ganglion cells with peripheral processes innervating receptor cells or structures and central fibers terminating in brain stem nuclei. To our knowledge, comparable studies that examine the central physiological effects of peripheral damage in mammals have only been accomplished in the somatosensory system. Peripheral nerve damage to spinal, visceral, or trigeminal nerves results in significant ganglion cell death (15–30%) and central transganglionic degeneration (Aldskogius et al. 1985). In the taste system, there is similar central degeneration but apparently little cell death after nerve transection in the middle ear (Whitehead et al. 1995). It is not clear why complete crush injuries (Cain et al. 1996) result in less complete regeneration than after transection (Whitehead et al. 1995) in this system, whereas in the somatosensory system, the opposite is true (Horch and Lисней 1981). In the CT-lingual mixed nerve of cats, crush injury has less severe effects than transection (Robinson 1989), but the crush injury may have been less complete than in our studies. In the somatosensory system, receptive fields of regen­erated fibers and their central target neurons are often not well preserved (Burgess and Horch 1973; Klein 1991; Koerber et al. 1995; Renehan et al. 1989). Receptive field properties were not examined in this study, but in a study of the cat chorda tympani-lingual nerve, gustatory receptive field sizes were unchanged after nerve crush and regeneration (Robinson 1989). Acute CT anesthesia in rats results in an increase in responsiveness of some NST neurons to stimulation of gustatory fields innervated by the glossopharyngeal nerve (Halpern and Nelson 1965). A recent study found such effects to be infrequent unless they considered the increased signal-to-noise ratio caused by a reduction in spontaneous rate (Dinkins and Travers 1998). However, after CT regeneration, we observed a recovery of normal spontaneous rates of NST units, whereas in the trigeminal system there are unexplained increases in the spontaneous rate of central neurons after nerve damage and regeneration (Klein 1991).

Of great relevance to the taste system is the ability of regenerated somatosensory fibers to contact the appropriate type of receptor and corresponding central neuron and to innervate the same proportion of various receptor types as normal. Infraorbital nerve transection and regeneration in rats results in a change in the proportion of nerve fibers responding to different types of stimuli (Renehan et al. 1989). A similar change in the gustatory system could account partially for the reduced signal-to-noise ratio caused by a reduction in spontaneous rate (Burgess and Horch 1973; Klein 1991; Koerber et al. 1995; Renehan et al. 1989). Receptive field properties were not examined in this study, but in a study of the cat chorda tympani-lingual nerve, gustatory receptive field sizes were unchanged after nerve crush and regeneration (Robinson 1989). Acute CT anesthesia in rats results in an increase in responsiveness of some NST neurons to stimulation of gustatory fields innervated by the glossopharyngeal nerve (Halpern and Nelson 1965). A recent study found such effects to be infrequent unless they considered the increased signal-to-noise ratio caused by a reduction in spontaneous rate (Dinkins and Travers 1998). However, after CT regeneration, we observed a recovery of normal spontaneous rates of NST units, whereas in the trigeminal system there are unexplained increases in the spontaneous rate of central neurons after nerve damage and regeneration (Klein 1991).

A 10% change in central convergence of peripheral functional fiber types would be difficult to detect in the gustatory system as there is considerable convergence normally.

An important finding of the present study was a reduction in taste-evoked net-spike rates after CT crush and regeneration, a finding that is consistent with a reduction in threshold. In the somatosensory system, such comparisons have not been emphasized. In the olfactory system, stimulus-response functions of some olfactory bulb neurons appear to completely recover after olfactory nerve section (Costanzo 1999), but olfactory-evoked potentials in the bulb did not completely recover (Koster and Costanzo 1996). The behavioral consequences of a reduction in evoked activity after regeneration appear small based on studies of nerve section or receptor sheet damage in the olfactory system (Yee and Costanzo 1995; Youngentob et al. 1997) and nerve crush or section in the taste system (Barry et al. 1993b; St. John et al. 1995) of rodents. This recovery may be partially due to central compensatory processes. However, different behavioral measures may reveal more subtle behavioral deficits.

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