Single Neurons in the Nucleus of the Solitary Tract Respond Selectively to Bitter Taste Stimuli

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Geran, Laura C. and Susan P. Travers. Single neurons in the nucleus of the solitary tract respond selectively to bitter taste stimuli. J Neurophysiol 96: 2513–2527, 2006. First published August 9, 2006; doi:10.1152/jn.00607.2006. Molecular data suggest that receptors for bitter ligands are coexpressed in the same taste receptor cells (TRCs), whereas physiological results indicate that individual TRCs respond to only a subset of bitter stimuli. It is also unclear to what extent bitter-responsive neurons are stimulated by nonbitter stimuli. To explore these issues, single neuron responses were recorded from the rat nucleus of the solitary tract (NST) during whole mouth stimulation with a variety of bitter compounds: 10 μM cycloheximide, 7 mM propylthiouracil, 10 mM denatonium benzoate, and 3 mM quinine hydrochloride at intensities matched for behavioral effectiveness. Stimuli representing the remaining putative taste qualities were also tested. Particular emphasis was given to activating taste receptors in the foliate papillae innervated by the quinine-sensitive glossopharyngeal nerve. This method revealed a novel population of bitter-best (B-best) cells with foliate receptive fields and significant selectivity for bitter tastants. Across all neurons, multidimensional scaling depicted bitter stimuli as loosely clustered yet clearly distinct from nonbitter tastants. When neurons with posterior receptive fields were analyzed alone, bitter stimuli formed a tighter cluster. Nevertheless, responses to bitter stimuli were variable across B-best neurons, with cycloheximide the most, and quinine the least frequent optimal stimulus. These results indicate heterogeneity for the processing of ionic and nonionic bitter tastants, which is dependent on receptive field. Further, they suggest that neurons selective for bitter substances could contribute to taste coding.

INTRODUCTION

Recent evidence suggests that compounds described as bitter by humans and avoided by rodents bind to members of a common group of receptors, the T2Rs (Adler et al. 2000; Matsunami et al. 2000). Individual T2Rs tested to date bind selectively to particular bitter stimuli or classes of structurally related bitter tastants (see Behrens et al. 2004; Bufe et al. 2005; Chandra shekhar et al. 2000; Pronin et al. 2004), suggesting the possibility that activation of one T2R could produce a different sensation of bitterness than activation of another T2R. Consistent with this idea, calcium imaging indicated that the majority (65%) of bitter-responsive taste receptor cells (TRCs) responded to only one of the five bitter stimuli tested (Caicedo et al. 1993). In addition, responses to various bitter stimuli did not correlate well with one another across single peripheral nerve fibers (Dahl et al. 1997). On the other hand, in situ hybridization demonstrated that individual T2R mRNAs are coexpressed in the same subset of TRCs, prompting the hypothesis that activation of these T2R-expressing TRCs yields a unitary sensation of “bitterness” (Adler et al. 2000). This hypothesis is supported by the behavioral literature because rats appear unable to discriminate between the two bitter stimuli quinine and denatonium (Spector and Kopka 2002). In addition to the question of whether all bitter compounds use the same coding mechanism(s), in the CNS it is also unclear whether bitter responses occur primarily in neurons broadly tuned to electrolytes (Di Lorenzo et al. 2003; Giza et al. 1991; Hasegawa et al. 2003; Lemon and Smith 2005; Lemon et al. 2003; Ogawa et al. 1987) or whether bitter-selective neurons constitute a distinct population of cells.

Few experiments have used bitter stimuli other than quinine, making it difficult to assess whether there is variation in how representatives of this taste quality are encoded. Perhaps attributable in part to this convention, most single-unit studies in the nucleus of the solitary tract (NST) have reported small numbers of neurons, typically <5%, that are optimally responsive to bitter tastants (reviewed in Spector and Travers 2005; Spector and Kopka 2002). In addition to the question of whether all bitter compounds use the same coding mechanism(s), in the CNS it is also unclear whether bitter responses occur primarily in neurons broadly tuned to electrolytes (Di Lorenzo et al. 2003; Giza et al. 1991; Hasegawa et al. 2003; Lemon and Smith 2005; Lemon et al. 2003; Ogawa et al. 1987) or whether bitter-selective neurons constitute a distinct population of cells.

The current study used whole mouth stimulation and stretched the foliate papillae open to ensure that tastants reached these posterior lingual TRCs (Halsell et al. 1993; Travers and Norgren 1995; Travers et al. 1986). In addition, electrode tracks extended more caudally and medially to GL-innervated regions of the NST (Hamilton and Norgren 1984) and bitter tastants other than quinine were periodically used as search stimuli. Finally, neurons were antidromically activated from the parabrachial nucleus (PBN) to provide evidence for potential contributions to ascending (discriminative) or local (reflective) pathways (Cho et al. 2002; Monroe and Di Lorenzo 1995; Ogawa and Hayama 1984; Ogawa et al. 1984a, 1987). These methods combined to reveal a higher proportion of
bitter-best (B-best) cells (14%). Most of these cells responded to foliate stimulation and were highly selective for bitter tastants. Furthermore, although B-best cells often responded to more than one bitter stimulus, bitter tastants varied in their effectiveness, suggesting the possibility of heterogeneous processing within this stimulus class. Portions of this paper were presented at the annual meeting of the Association for Chemo-reception Sciences (AChemS) in Sarasota, FL, April 2005.

METHODS

Subjects

Data were collected from 73 adult male Sprague–Dawley rats (Harlan) weighing 250–500 g. All manipulations were performed according to the guidelines of the Institutional Laboratory Animal Care and Use Committee (ILACUC) at the Ohio State University.

Surgery

Animals were deeply anesthetized with Inactin (thiobutabarbital sodium, 100 mg/kg, administered intraperitoneally [ip]) and maintained at this level with Nembutal (sodium pentobarbital, 20 mg/kg, ip). Body temperature was kept at about 37°C during the procedure by means of a heating pad. The hypoglossal nerve and superior laryngeal branch of the vagus nerve were bilaterally transected and a polyethylene tracheal tube was inserted to aid breathing. In addition, to provide access to the oral cavity, sutures were placed in the lips and tongue as described previously (Halsell et al. 1993; Norgren et al. 1989; Travers et al. 1986). Of particular importance, one suture was inserted just under the oral mucosa and slightly anterior to the foliate papillae, so that the tongue could be pulled anteriorly and laterally to expose and open the foliate trenches. Rats were placed in a nontraumatic headholder and the skin and fascia on the top of the head were dissected away from the bone. Two holes were drilled in the skull, one just lateral to lambda in the parietal bone anterior to the PBN and a second that removed most of the interparietal bone to allow access to the NST. A skull screw was inserted in the parietal bone anterior to the first hole to provide an anchor for cementing the PBN electrode.

The “waist region” of the PBN was located using a commercial tungsten microelectrode (200 kOhm to 1 MOhm, FHC) inserted about 0.2 mm rostral and 1.8 mm lateral to lambda. The electrode was inserted at a 20° angle to avoid the transverse sinus and driven down 6–7 mm while monitoring the response to the search mixture with an oscilloscope and an audio monitor. If the activity increased in response to the mixture but not to distilled water over a distance of about 0.5 mm, the electrode was considered to be in the “waist.” After locating this region, a concentric bipolar electrode (0.5-mm separation, 50–250 kOhm) was inserted in the same location under visual and electrophysiological guidance and fixed in place with dental acrylic. The inner pole of this electrode consisted of insulated stainless steel wire with a diameter of 200 μm (125 μm uninsulated). The outer pole was 26 Ga stainless steel tubing insulated with Epoxylite 6001 except for the tip (0.25 mm).

Once the stimulating electrode was fixed, the NST was located using a low-impedance (<1 MOhm) search electrode. The search electrode was then replaced with a higher-impedance recording electrode (1–5 MOhm, FHC or World Precision Instruments) to isolate single units. To find the NST, the search electrode was initially inserted about 4.5 mm caudal and 1.8 mm lateral to lambda. Typically, the rostral NST was located 5–6 mm ventral to the surface of the cerebellum and was marked by a decrease in spontaneous multi-unit activity coupled with increased activity in response to the search mixture. After recording from a unit, a small lesion was usually made either at the site or just under the NST on the same track. We attempted to sample from as much of the taste-responsive area of the NST as possible, including regions that received projections primarily from the GL nerve (see Hamilton and Norgren 1984; Travers et al. 1986). On completion of recording, each subject was given an overdose of sodium pentobarbital and perfused with saline and 10% buffered formalin. Brains were extracted and refrigerated in 20% sucrose-formalin. At least 24 h later, the brains were sectioned at a thickness of 52 μm using a freezing microtome and alternately stained with Weil and Cresyl.

Search and stimulation protocols

To locate the taste-responsive regions of the PBN and NST a search mixture consisting of 20 mM Na saccharin, 100 mM NaCl, 10 mM HCl, 30 mM monopotassium glutamate (MPG), 3 mM inosine monophosphate (IMP), and 3 mM quinine hydrochloride (QUI) was introduced into the mouth by way of a syringe. Occasionally, one or more of the following stimuli were also used to probe the NST for cells that failed to respond to the search mixture: 2.5 M KCl, 7 mM propylthiouracil (PROP), 10 mM dinitonium benzoate (DEN), 10 μM cyclic AMP (cAMP), or 30 mM QUI. After a single taste-responsive neuron was isolated, stimuli were delivered by way of a pressurized flow system controlled by Spike2 software (Cambridge Electronic Design), with a mouthpiece oriented to achieve whole mouth stimulation similar to that reported by Baird et al. (2001). Visual inspection revealed that the fluid flowed over all gustatory receptor populations including the VIIth nerve-innervated anterior tongue, nasoniocinor ducts, and soft palate, as well as the IXth nerve-innervated foliate and circumvallate regions. As mentioned above, a suture was used to stretch the foliate trenches open and optimize access to the taste receptor cells in these papillae. The length of the protocol, however, did not allow us to use a second mouthpiece to optimize stimulation of the circumvallate, which requires that fluid be delivered directly to the trench surrounding the papilla. Altogether, we achieved effective stimulation for all of the VIIth nerve-innervated taste buds, and also for 57% (Travers and Nicklas 1990) of the IXth nerve-innervated taste buds on the posterior tongue. The flow rate was about 2 μl/s. Each of the 13 automated trials [12 stimuli (see following text) + search mixture] lasted 1 min and consisted of 10 s of spontaneous activity, 10 s of distilled water, 10 s of taste stimulation, 20 s of distilled water rinse, and another 10 s of spontaneous activity. The intertrial interval was about 1 min.

If a neuron remained isolated following characterization of whole mouth taste responsiveness, the gustatory receptive field (RF) was assessed and the cell was also tested to determine whether it could be antidromically activated from the PBN. The RF for each unit was tested by stroking the anterior tongue (AT), nasoincisor ducts (NIDs), circumvallate regions. As mentioned above, a suture was used to stretch the foliate trenches open and optimize access to the taste receptor cells in these papillae. The length of the protocol, however, did not allow us to use a second mouthpiece to optimize stimulation of the circumvallate, which requires that fluid be delivered directly to the trench surrounding the papilla. Altogether, we achieved effective stimulation for all of the VIIth nerve-innervated taste buds, and also for 57% (Travers and Nicklas 1990) of the IXth nerve-innervated taste buds on the posterior tongue. The flow rate was about 2 μl/s. Each of the 13 automated trials [12 stimuli (see following text) + search mixture] lasted 1 min and consisted of 10 s of spontaneous activity, 10 s of distilled water, 10 s of taste stimulation, 20 s of distilled water rinse, and another 10 s of spontaneous activity. The intertrial interval was about 1 min.

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Taste stimuli

All stimuli were purchased from Sigma-Aldrich (St. Louis, MO) except NaCl and sucrose (Fisher Scientific, Fair Lawn, NJ). Test stimuli consisted of two sweeteners (300 mM sucrose and 300 mM sucrose-formalin. At least 24 h later, the brains were sectioned at a
to deliver fluid to the oral cavity and subtracted this amount from each latency value before statistical analysis.

All statistics were performed using Systat 10 (SPSS). These included one-way ANOVAs, paired- and two-group t-tests, and $\chi^2$ analyses. The alpha value was set at 0.05 for each. In addition, as described in more detail below, across-neuron correlations, multidimensional scaling, and hierarchical cluster analysis were performed.

Pearson’s $r$ values (i.e., “across-neuron correlations”) were calculated between each stimulus pair across cells to quantify the similarities in relative rates of activity evoked across the sample of recorded neurons, i.e., “across-neuron patterns” (Erickson 1963). A multidimensional scaling (MDS) procedure was then executed using these across-neuron correlation coefficients. In the MDS, the degree of similarity among the across-neuron patterns evoked by the 12 stimuli was graphically depicted by plotting the stimuli as a function of their similarity to one another in an $n$-dimensional space. In neurophysiological studies of taste processing, qualitatively similar taste stimuli (e.g., sucrose and fructose) typically elicit more similar across-neuron patterns than qualitatively distinct stimuli (e.g., sucrose and HCl; Bieber and Smith 1986; Erickson et al. 1993; Schifman et al. 1981).

Hierarchical cluster analysis was used to place neurons into groups, or clusters, according to their relative response rates across tastants (Bieber and Smith 1986). For each neuron pair, the similarity in responses across the 12 stimuli was quantified by calculating Pearson’s $r$. The dissimilarity (or distance) between pairs could then be expressed as $1 - r$. The resulting distance matrix was the basis for the stepwise process of combining each neuron with the next most similar cell, or group of cells, until all neurons were combined into one cluster. The method of average linkage was used for this process. Hierarchical cluster analysis produced a cluster tree, or graphical representation, of these stepwise combinations (Fig. 3B). A score plot depicting the distance at each step of the clustering process was also generated from these data to help determine the number of meaningful clusters (see Fig. 3A). Customarily, the first breakpoint in the score plot, which indicates that further amalgamation notably increases the distance between combined cells, serves as the main criterion for defining groups (Bieber and Smith 1986). In addition to this criterion, we also required that there be a minimum of three cells per group and that the average correlations between response profiles in all groups equal or exceed a statistically significant value [$r_{0.05,10} = 0.576$ for 12 stimuli].

Entrophy ($H$) values were determined using the following equation to quantify the breadth of tuning (Smith and Travars 1979)

$$H = -K \sum_{i=1}^{K} P_i \log P_i$$

where $K$ represents a constant and $P_i$ is the proportional response attributed to each stimulus class. Because each taste category consisted of more than one stimulus (i.e., “sweet” = sucrose and glycine), $P_i$ was determined using the average for each taste quality. Negative averages were replaced with zeroes and $P_i$ values of zero were replaced with $0.001$ to avoid undefined entropy results. Entropy was determined using five (sour, salty, sweet, bitter, and umami) taste qualities with $K$ equal to 1.43 for five classes. An entropy score ($H$) near zero indicates narrow tuning (i.e., a cell responded only to stimuli of a particular taste quality), whereas a score approaching unity indicates broad tuning (Smith and Travars 1979). However, because the existence of the umami quality is still somewhat controversial, all analyses that compare $H$ scores between neurons were also calculated with the classic four qualities. Because the two analyses agreed on the vast majority of occasions, only the $H$ values for five qualities are reported here.

Electrophysiological data analysis

There were two criteria for a response. A significant response was defined as one in which the number of spikes during taste stimulation minus the number of spikes during the preceding water stimulation was $I > 2.5$ times the SD for distilled water across all trials (see Nishijo et al. 1991) and 2) exceeded 1 spike/s; i.e., a net response of $\geq$10 spikes in the 10-s stimulation period. If a stimulus was tested more than once, the mean was used for analysis. There were 137 responses that met the first part of the criterion but four of these cells had response rates of $<1$ spike/s and so were not included in the analyses, resulting in an $N$ of 133.

Waveforms were analyzed off-line and spikes counted using Spike2 software. Some neurons, particularly those responsive to bitter tastants and posterior oral cavity stimulation, did not appear to reach their peak firing rate as quickly as the other cells. To assess this systematically, we analyzed responses to tastants for each cell’s best stimulus category ($n = 183$ responses). Spike rates were summed across 1-s bins and the time until peak response noted. In cases where the peak encompassed two or more bins, the average latency was used. In four cases (all B-best), an even larger response occurred during the rinse phase of the trial, suggesting that the latency of the true peak taste response might have been $>10$ ms for these cells, although peaks within only the 10-s taste phase of the trial were used in the analysis. We determined that the flow system required 680 ms

glycine), two salts (100 mM NaCl and 100 mM Na gluconate, or NaGlu), two acids (10 mM HCl and 30 mM citric acid), two glutamate (“umami”) stimuli [30 mM MPG + 3 mM IMP and 30 mM monosodium glutamate (MSG) + 3 mM IMP], and four bitter stimuli (3 mM QUI, 7 mM PROP, 10 mM DEN, and 10 mM CHX). IMP, a nucleotide that acts synergistically with glutamate stimuli (Delay et al. 2000; Yamamoto et al. 1991), was used in combination with MSG and MPG to maximize responses from glutamate-sensitive cells so that they might be more easily discriminated from neurons that responded best to salts or sweeteners. For the four “prototypical” taste stimuli (sucrose, NaCl, HCI, QUI), it was possible to choose test concentrations that represented midrange (roughly half-maximal) afferent inputs to the NST, based on peripheral whole-nerve recordings (Frank 1968; Nejad 1986; Ogawa 1972; Pfaffmann et al. 1967). Because the nerves that innervate different taste receptor subpopulations exhibit some specialization for different categories of tastants, when the midrange concentration varied between nerves, the intensity was chosen based on the nerve most responsive to a given tastant (NaCl: chorda tympani; sucrose: greater superficial petrosal, QUI: glossopharyngeal). Because MSG and IMP are not widely used in whole nerve recordings, the MSG + IMP concentration was based on a behavioral criterion, i.e., the peak of the preference function (Delay et al. 2000).

Concentrations of citric acid and glycine were chosen so that they activated brain stem neurons to a similar degree as the midrange concentrations of HCl and sucrose, based on earlier recordings from the NST and PBN (see Baird et al. 2001; Nishijo et al. 1991; Travers and Norgren 1991). The NaGlu and MPG concentrations were equimolar to NaCl and MSG, respectively. Behavioral criteria were also used to pick the additional bitter stimulus concentrations. The DEN concentration produced results similar to 3 mM QUI both in unconditioned avoidance and taste reactivity experiments (see Chan et al. 2004; Spector and Kopka 2002) and the PROP concentration was comparable to 10 mM DEN and 3 mM QUI in a taste reactivity study (Chan et al. 2004). Because neither comparable behavioral nor neural data were available for CHX, the concentration (10 mM) was chosen based on its ability to activate rat TRCs (Caicedo and Roper 2001). As described below, however, CHX most often proved to be the optimal stimulus for B-best cells and we became concerned that this might be explained by superior behavioral effectiveness. We subsequently assessed a range of CHX concentrations for effectiveness in eliciting the oral rejection response (see Taste reactivity below for details).
Taste reactivity

Five rats were fitted with intraoral cannulae as described in Chan et al. (2004). After a 2- to 5-day recovery period, animals were given a 1-h habituation session followed the next day by a 30-min water adaptation session during which distilled water was delivered several times through each cannula. A Spike2 program controlled fluid delivery by a pressurized flow system calibrated to deliver 50 μL of fluid in 1.4 s. Each trial was composed of 15 s of no stimulation, 1.4 s of taste stimulation, a 60-s waiting period, and then six 1.4-s, 50-μL distilled water rinses delivered 5 s apart, followed by an intertrial interval of ≥1 min. Animals were filmed using a digital camera and the video data were synchronized with fluid onset and offset using Spike2.

The day after water adaptation (Day 1), each rat was tested with 3 mM QUI and 0.3 M sucrose; on Day 2, two concentrations of CHX in ascending order (3 and 10 μM); and on Day 3, 30 μM CHX. Day 4 was a repeat of Day 1. Distilled water trials were interspersed with gustatory stimuli to lessen potentially aversive associations with the testing chamber. The number of gapes during the first 10 s after fluid delivery was counted, and all aversive behaviors including gapes, chin rubs, and passive dripping episodes occurring in the 40-s period after fluid delivery were also tabulated. These analyses revealed that CHX (10 μM) elicited roughly the same number of gapes in 10 s [paired t(4) < 0.12] and total aversive responses in 40 s [t(4) < 1.6, P > 0.17; see Supplemental figure1] as 3 mM QUI. Differences between the first and last day of QUI testing were not significant. These results suggest that 10 μM CHX is comparable in behavioral potency to the other three bitter stimuli used in the electrophysiological experiment.

RESULTS

Overall characteristics

A total of 133 gustatory neurons fit the response criteria and were tested with the full array of taste stimuli. The majority were isolated for a period of time that also allowed further testing to assess their receptive field (n = 120) and to determine whether they could be antidromically activated by PBN stimulation (n = 110). The mean (±SD) spontaneous rate for all cells in the experiment was: 2.1 ± 0.3 spikes/s. As mentioned earlier, a considerable effort was made to record from neurons responsive to posterior oral cavity stimulation. Consistent with our previous experience (see Halsell and Travers 1997; Travers and Norgren 1995), however, these cells were more difficult to isolate and more neurons responsive to anterior oral cavity stimulation were amassed. Nevertheless, of cells with known RFs (n = 120), 26% (n = 31) were in the posterior oral cavity (PO; soft palate and/or foliate papillae). The remaining 72% (n = 86) were located in the VfTh nerve-innervated anterior oral cavity (AO), anterior tongue, and/or nasoincisor ducts. A majority of PO cells likely received input from the IXth nerve given that 74% (23 of 31) had some portion of their RF on the foliate papillae. Three additional cells (2%) responded well to stimulation of both AO and PO regions.

AO and PO units were associated with different chemosensitive profiles, i.e., neurons responding optimally to bitter stimuli most often received input from the PO (see following text). PO units were also more narrowly tuned (mean H value ± SD = 0.43 ± 0.25) than those receiving input from the AO (H = 0.69 ± 0.13; t = 7.2, P < 0.001). None of the neurons in the present study responded to attempts at gustatory stimulation of the circumvallate papilla during RF testing, although 13 cells had unknown RFs, making it possible that these units received circumvallate input. Also, units with known RFs, particularly those with foliate responses, might have received convergent input from the circumvallate (see Halsell et al. 1993). Given the difficulty involved in stimulating TRCs in the circumvallate trench of the rat (Frank 1991; Halsell and Travers 1997), however, these inputs most likely remained inactive throughout the experiment.

Antidromic activation from the PBN

Of the 110 cells (37%) tested, 41 were antidromically activated by PBN stimulation. Several response properties were related to whether a cell projected to the PBN. Consistent with previous reports (Monroe and Di Lorenzo 1995; Ogawa et al. 1984b), neurons antidromically activated by PBN stimulation responded more vigorously to gustatory stimulation than those not antidromically activated, as assessed by firing rates evoked by the stimulus eliciting the optimal response for a given cell (mean ± SE: 221 ± 195 vs. 121 ± 134; t = 3.3, P < 0.002). Furthermore, antidromically activated neurons were more broadly tuned (H = 0.67 vs. 0.56, t > 2.2, P < 0.03), and constituted a larger percentage of AO (47%; n = 34/73) than PO (19%; n = 5/26) cells (χ² = 6.0, P < 0.02; Table 1A). The PO units activated by PBN stimulation had longer antidromic latencies than comparable AO neurons, suggesting slower conduction velocities (Fig. 1A). Furthermore, neurons maximally activated by bitter tastants (see following text) had longer latencies than cells maximally responsive to other stimuli (Fig. 1B).

Representation of taste stimuli across neurons

The similarity in the relative rates of response evoked across NST neurons by each pair of stimuli was assessed by calculating the Pearson’s correlation coefficient (r) (“across-neuron correlations”; Table 2). Similarities among these “across-neuron patterns” were summarized graphically using multidimensional scaling (MDS, Fig. 2A). Based on previous work (see Bieber and Smith 1986; Erickson et al. 1993; Schiffman et al. 1981), it was expected that higher correlations, indicative of more similar across-neuron patterns would be obtained between the qualitatively similar taste stimuli than between qualitatively dissimilar pairs. Except for the relationships between bitter stimuli, the predicted results were obtained. There were high and significant across-neuron correlations (+0.86 to +0.94) between stimuli from qualitatively similar pairs; i.e., sucrose and glycine (“sweet”), NaCl and NaGlut (“salty”), HCl and citric acid (“acid”), and MSG and MPG (“umami”). In contrast, correlations between these distinct stimulus categories were lower (+0.06 to +0.78). As a consequence, in the MDS plot, the two stimuli within each qualitatively similar pair were close to each other, but the pairs were segregated, albeit to varying degrees.

As expected, correlations between each of the bitter tastants (QUI, DEN, PROP, CHX) and the other stimuli were also low (−0.36 to +0.47) and in the MDS plot, the bitter stimuli were segregated from salty, sour, sweet, and umami tastants. Contrary to what would be predicted if bitter stimuli were processed as a homogeneous group, however, correlations be-

1 The online version of this article contains supplemental data.
Fig. 1. Scatterplots with antidromic latency on the x-axis and maximum response rate (spikes/10 s) on the y-axis for each of the 41 nucleus of the solitary tract (NST) neurons antidromically activated from the parabrachial nucleus (PBN). Left panel (A): cells coded for receptive field (RF). Right panel (B): cells coded for chemosensitive profile (i.e., cluster). A: RFs in the anterior oral cavity are represented with white circles (n = 34), posterior RFs with black (n = 5), mixed RFs with gray (n = 1), and unknown RFs with gray squares (n = 1). B: bitter-best (B-best) units are shown in black; cycloheximide (CHX) cells (circles, n = 2) and denatonium benzoate (DEN) units (squares, n = 2). Electrolyte-sensitive cells are shown in gray; sodium salt (N) units (circles, n = 14) and acid and sodium salt (A/N) units (squares, n = 8). Sucrose-responsive neurons are in white; amino acid and sucrose (U/S) cluster (circles, n = 14); and sucrose (S) cluster (squares, n = 1). Note that bitter cluster cells (black symbols, right) and those with posterior RFs (black symbols, left) have longer latencies (≥10 ms).
Classification of neurons by chemosensitive profile

Hierarchical cluster analysis was used to classify neurons according to their relative responsiveness to different tastants. Preliminary analysis indicated that 8/133 neurons (6%) could not be assigned to groups and so these neurons were removed from the cluster tree for clarity; however, the same groups were identified irrespective of whether the outliers were included.

The cluster tree and scree plot for the remaining 125 neurons suggested six groups (Fig. 3). At the initial stages of amalgamation the distance \(1/r\) between neuron profiles increased incrementally but after six groups were defined, it increased sharply (arrow, scree plot; dotted line on cluster tree). In addition, if amalgamation was continued, the resulting group would have contained neurons with profiles that correlated at \(r = 0.46\) and did not meet the additional criterion for a statistically significant correlation between neurons in a group (i.e., \(r = 0.58\); see METHODS).

The response profiles for the six groups are shown in Figs. 4 (mean responses) and 5 (individual responses). Notably, there were two clusters of narrowly tuned bitter-best (B-best) neurons, including a smaller group optimally responsive to \(\text{DEN (n = 3)}\) and a larger group maximally responsive to \(\text{CHX}\).

### Table 2. Across-neuron correlations

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Significant correlations are underlined (\(r > 0.576; \alpha < 0.05\)). Correlations within a putative stimulus quality are in bold. Correlations with bitter stimuli are indicated by background shading. \(n = 133\).

**FIG. 2.** A: 2-D multidimensional scaling (MDS) plot for all units. B and C: MDS broken down by RF (posterior on the left and anterior on the right). Stimuli of the same putative taste quality are represented with like symbols (e.g., “sour” stimuli with open circles, etc.). Note the tighter clustering of bitter stimulus responses among cells with RFs in the posterior oral cavity (B). Those with more anterior RFs (C) formed 2 clusters for bitter stimuli: one for the ionic stimuli quinine hydrochloride (QUI) and DEN, which correlated better with the salts and acids, and one for the nonionic bitters propylthiouracil (PROP) and CHX, which was more segregated from the nonbitter stimuli.
Other groups were optimally responsive to sucrose (S, n = 8), amino acids and sucrose (U/S, n = 36), acids and sodium salts (A/N, n = 37), or sodium salts (N, n = 31).

Response profiles of the eight outlier neurons were heterogeneous, but five responded optimally to one of the bitter tastants. Because we were particularly interested in bitter responsiveness, response profiles for these unclustered bitter-best cells are also shown in Figs. 4 and 5.

Chemosensitive response profiles were related to receptive field. Overall, more neurons had AO receptive fields but a greater number of S and B-best neurons had PO receptive fields ($\chi^2 = 37.8, P < 0.001$; Table 1B). In addition, when clusters were collapsed into two groups based on hedonic quality (U/S, S, and N neurons that responded optimally to sweeteners, glutamate, or sodium vs. A/N and B-best neurons that responded best to acids or bitters), a relationship to antidromic activity by PBN stimulation emerged ($\chi^2 = 4.1, P < 0.05$). Only 27% of cells (n = 12 of 45) optimally responsive to avoided stimuli could be antidromically invaded from the PBN compared with 47% (n = 29 of 62) of those optimally responsive to preferred stimuli (Table 1C).

Detailed response properties of chemosensitive groups

BITTER-BEST (B-BEST) NEURONS. In the cluster analysis (Fig. 3), B-best neurons were combined with the other groups of cells only at the final stage of amalgamation. These cells overwhelmingly received input from the PO (12 of 14 neurons with known RFs, Table 1B).

DEN and CHX cluster neurons were both narrowly tuned to bitter stimuli (Figs. 4 and 5); i.e., they responded minimally if at all to other taste qualities. For the CHX group, the entropy value (0.32 ± 0.07) was significantly lower than that for the U/S (0.65 ± 0.03), N (0.67 ± 0.03), or A/N-groups (0.61 ± 0.04; P < 0.002 for each). The entropy value for DEN units (0.37 ± 0.06) was almost identical to that for CHX cells.

Although cells in both the CHX and DEN clusters were narrowly tuned across qualities, they differed with regard to
responsiveness across bitter stimuli. Aside from the difference in optimal stimulus, DEN neurons exhibited a rather specific sensitivity to DEN, whereas CHX neurons tended to be more broadly responsive across bitter tastants (Figs. 6 and 7). Nevertheless, even within the CHX cluster, responsiveness to bitter tastants was not equivalent. Notably, the response to QUI was small, on average only 26% as large as the CHX response (Fig. 4); indeed only one CHX neuron responded even half as well to QUI. In addition, on average, CHX cells responded well to both DEN and PROP (Fig. 4), although the PROP response of individual neurons was variable, ranging from a small decrement to a firing rate that was >75% that elicited by CHX (Figs. 5 and 7). The differential responsiveness to bitter tastants in DEN and CHX neurons probably contributes to the dissimilar across-neuron patterns discussed above (Table 2, Fig. 2).

Five additional B-best neurons failed to cluster (Unclustered B-best cells, Figs. 4 and 5). Compared with DEN and CHX neurons, unclustered B-best cells exhibited greater sideband responses to nonbitter stimuli. This was reflected in an average entropy value (0.70 ± 0.11) roughly twice that of the DEN and CHX clusters (two-group t > 2.7, P < 0.04 for CHX vs. unclustered). Because responses from unclustered B-best cells were idiosyncratic, however, the mean response profile (Fig. 4) suggested more specific tuning for bitter versus other qualities than was evident in individual neurons (Fig. 5). Overall, the five unclustered B-best cells were distinguished by a relatively small CHX response coupled with significant responses to nonbitter stimuli.

It should be emphasized that responses to the prototypical stimulus, quinine, were modest in all B-best cells (CHX, DEN, or unclustered). Paired t-tests (n = 18) indicated that responses to QUI were significantly smaller than those to other bitter stimuli (P < 0.02 for each). The other bitter stimuli were not different from one another (P > 0.14) and mean responses to QUI were comparable to HCl (22.8 vs. 9, P > 0.13) but greater than NaCl (22.8 vs. 0.6, P < 0.03). It should likewise be emphasized that, although B-best neurons demonstrated heterogeneity in bitter responsiveness, this was not indicative of a lack of response reliability. Stimulus trials were repeated on 51 occasions in 13 B-best neurons and the responses were highly correlated (r = +0.90).

**OTHER NEURONS.** A characteristic common to neurons with optimal responses to sodium, acids, sugar, or glutamate was their relatively small response to bitter compounds when compared with that of other tastants. This is apparent not only in the mean response profiles (Fig. 4), but also in the responses of individual neurons (Fig. 5). S and U/S neurons exhibited virtually no responses to any of four bitter tastants. Bitter responses in N and A/N neurons were more evident, but still just a small fraction of the responses elicited by the optimal stimuli for those classes of cells. For example, QUI elicited a mean response just 12% as great as the response to NaCl in N neurons. Interestingly, only certain bitter compounds—QUI and DEN—evoked even small responses in non-B-best neurons. This is particularly obvious in Fig. 5, which shows that, even in the N and A/N neurons with the largest responses to QUI or DEN, PROP and CHX elicited virtually no activity and, in some cases, elicited an inhibitory response. These sideband responses to DEN, and particularly QUI in N and A/N neurons, coupled with QUI’s poor effectiveness in the B-best neurons, apparently provide the basis for the position of these stimuli in the MDS plot (i.e., midway between CHX and PROP and the nonbitter electrolytes).

Some clusters of neurons in the present study were similar to those described previously. For instance, a large N cluster, maximally responsive to sodium, was found, as well as a group of A/N cells more broadly tuned to sodium and acids (e.g., NaCl-best: Lundy and Norgren 2001; N-cells: McCaughey and Scott 2000; NaCl-best: Ogawa et al. 1984b; N-best: St John and Smith 2000). The majority of N and A/N units had receptive fields in the AO (54/64 tested).

Other neurons identified in the present study have been less evident in past studies, perhaps because they tended to have PO

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**FIG. 4.** Mean responses (±SE) across stimuli for each of the 6 clusters and for the bitter-best cells that failed to cluster (n = 5).
receptive fields. S neurons \((n = 8)\) were notable because almost all had PO RFs \((5/6)\) and because they were narrowly tuned to sweeteners. The entropy value was significantly lower among cells in the S cluster \((H = 0.32)\) than for the U/S \((H = 0.65)\), A/N \((H = 0.61)\), or N \((H = 0.67)\) groups \((\text{ANOVA}s, P < 0.001, \text{post hoc comparisons, all values of } P < 0.001)\). Similarly, a subcluster of the A/N neurons \((a_{A/N})\) was narrowly tuned to acids \((\text{Figs. 3 and 5, second group of plotted A/N cells})\). Like the narrowly tuned S neurons, these \(a_{A/N}\) cells were more likely to have PO inputs \((7/16)\).

Few previous CNS studies have used glutamate as a tastant and novel observations about umami responses also emerged. Many neurons, mainly with AO receptive fields \((30/35 \text{ cells})\), responded robustly to both amino acids and sweeteners \((U/S, \text{Fig. 4})\). The mean response to NaCl was just 40% of that to MSG, even though the NaCl concentration was threefold higher. In addition, responses to MSG and MPG were of similar magnitudes, suggesting that the glutamate anion, instead of the cation, was critical for activating these neurons. The U/S cluster included two subgroups \((\text{Figs. 3 and 5})\), reflecting variation in the relative responses to sweeteners and glutamate. The first subcluster \((\text{leftmost U/S cells, Fig. 5})\) included neurons that responded nominally better to sucrose \((n = 10)\) or glutamate \((n = 5)\), but responded similarly to the two stimuli \((\text{i.e., } < 25\% \text{ difference between the mean responses})\), indicating broader tuning. The \(u_{U/S}\) subcluster \((\text{rightmost U/S cells, Fig. 5})\) contained only U-best units \((n = 21)\) that, on average, responded twice as well to glutamate as to sweeteners or electrolytes, indicating more selective responding.

**RESPONSE TIME COURSE.** B-best cells shared some attributes with those in the S cluster in that both groups had low maximum response rates \((\text{Figs. 4 and 5})\) and a slow time
course. The time course differences appeared related to the
tendency for S, CHX, and DEN cluster cells to have PO RFs.
Responses from PO units exhibited a longer time to peak firing
rate (mean ± SE = 4.8 ± 2.3 s) than AO units (3.1 ± 2.3, t =
4.7, P < 0.001), suggesting that stimuli may take more time to
reach TRCs buried in the trenches of the foliate papillae. A
one-way ANOVA for latency to peak was also significant for
reach TRCs buried in the trenches of the foliate papillae. A
4.7, P

HISTOLOGY. Figure 8 depicts locations of the recorded cells in
coronal (Fig. 8A) and horizontal (Fig. 8B) planes. A concerted
effort was made to sample over a wide extent of the nucleus,
particularly in medial and caudal regions where GL input is
densest and representation in electrophysiological recording stud-
ies is less common. We had some success in isolating neurons
over this entire area, but as in previous studies, success rates were
higher at the rostral–lateral pole. Average coordinates of neurons
were calculated in the three anatomical axes. Anteroposterior
(AP) coordinates were expressed as the proportion of the distance
from the rostral pole to the level at which the NST merges with the
IVth ventricle, mediolateral (ML) coordinates were the proportion
of the distance from the lateral to the medial border, and dorso-
ventral (DV) coordinates were the proportion of the distance from
the dorsal to the ventral border. Consistent with previous reports,
there was an orotopic arrangement. Neurons with AO receptive
fields were typically anterior (25.1 vs. 32.8%); P < 0.03) and
medial (32.7 vs. 43.2%; P < 0.03) to those with PO receptive
fields; however, DV coordinates for the two groups were virtually
identical (49.1 vs. 48.5%; P > 0.1). B-best neurons were further
medial (45.8 vs. 34.8%; P < 0.05) and dorsal (20 vs. 52.5%; P <
0.004) than other cells. Their location along the rostral–caudal
axis, however, was not significantly different (29.1 vs. 27.5%;
P > 0.1). The location of B-best neurons in the dorsolateral axis
is consistent with results reported by Halpern (1965) that multiunit
QUI responses were somewhat larger in the dorsal part of the
nucleus. In addition, this dorsal–medial tendency among B-best
neurons is broadly consistent with observations from Fos immu-
nohistochemistry (Chan et al. 2004; King et al. 1999; Travers
2002). However, when the nucleus was divided into subfields
(dotted lines in Fig. 8A), few taste neurons of any type were
located in the dorsal–medial subfield where bitter-Fos neurons
were maximal.

Thirty-two parabrachial stimulation sites were reconstructed
(Fig. 9). Nineteen resulted in antidromic activation of at least
one gustatory cell. All placements were within 500 μm of the

FIG. 6. Representative bitter cell re-
ponses. A: responses from 2 cells to CHX.
Cell on top is a CHX cluster cell, whereas
the cell on the bottom is selective for DEN.
Vertical cursors indicate the 5 phases of each
trial: 10 s, spontaneous activity; 10 s, dis-
tilled water; 10 s, taste stimulus; 20 s, water
rinse; and 10 s, spontaneous activity. A his-
togram of the neural activity is located above
each record. B: responses from the same 2
cells to DEN. For the DEN-selective cell, the
negative-going potential was at saturation
for the AD converter.

FIG. 7. Stacked bar graph depicting responses to the 4 bitter stimuli in
B-best cells as a percentage of the total bitter response for each neuron. Only
significant responses were included. Individual cells are grouped by cluster
[left to right: CHX-best cells (n = 10), DEN-best (n = 3), and Unclustered
(n = 5)]. Note that very few cells responded equally to the 4 bitter stimuli.

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waist area and included placements in the cell bridges within the brachium conjunctivum, centromedial nucleus (CM), ventromedial nucleus (VM), external lateral (EL) nucleus, and mesencephalic nucleus of V (Me5; Paxinos and Watson 2004). Given that the separation between the poles was 500 μm and the width of each pole was about 200 μm, nearby areas were probably also stimulated. With the exception of EL, stimulation sites in the PBN that effectively drove antidromic responses in the NST also failed to drive activity. This suggests that failure to stimulate brain stem neurons from the PBN was a result of the projection status of the neuron and not the stimulating electrode location.

Stimulating electrode placements for three of the four B-best relay cells were reconstructed. One was located in the caudal CM ventral to the brachium, one in Me5, and one in the VM just lateral to Me5. Electrodes for four B-best cells that did not project to the PBN were located in the brachium, VM, and the ventral CM. Again, this suggests that PBN electrode placement did not affect antidromic classification.

Discussion

Overview

This paper characterizes a novel group of rat NST neurons selective for bitter stimuli that receive input primarily from TRCs in the foliate papillae. DEN and CHX cluster B-best cells were largely unresponsive to stimuli representing nonbitter taste qualities and unlike cells in other best-stimulus categories, often responded significantly only to a subset of tastants in their own best-stimulus group (Figs. 6 and 7). Quinine was particularly ineffective. Although the GL nerve is highly sensitive to QUI (Frank 1991), recent reports suggest that this nerve is even more responsive to certain other bitter stimuli, including CHX (Damak et al. 2006; Danilova and Hellekant 2003). This could explain why CHX was a more effective stimulus than QUI for B-best units in the current study, a result consistent with that of Caicedo and Roper (2001) at the level of the taste receptor cell. The QUI insensitivity of these B-best cells coupled with poor responses to nonbitter stimuli, low spontaneous firing rates, difficulty in stimulating foliate TRCs, and location in the NST most likely combine to account for why these neurons were not described previously.

The large size of the T2R family and robust expression of these receptors in the circumvallate papilla (Adler et al. 2000) suggest that incorporating even more varied bitter tastants and targeting this region of the posterior tongue will be important in fully defining bitter responsiveness. Similarly, a recent study in the NST (Di Lorenzo and Victor 2003) suggests that application of stimuli over multiple trials would provide added statistical power for defining gustatory response profiles.

Glutamate responses

Our data provide new information that suggests that some NST neurons are maximally activated by glutamate. Neurons in the u_h5 subcluster responded about twice as well to glutamate (e.g., MPG and MSG) as to sucrose and electrolytes, and constituted a significant proportion of the neurons within the group of cells broadly responsive to sucrose and glutamate (see Fig. 5). Although the cluster analysis failed to provide evidence that these cells formed a distinct group, the glutamate-best neurons probably contributed to the segregation of amino acids, sweeteners, and salts observed in the MDS plot (Fig. 2). Nishijo et al. (1991) previously reported glutamate-responsive neurons in the PBN but glutamate-best neurons were less apparent than in the current experiment, perhaps as a consequence of further convergence from the NST to PBN.
Representative pontine sections are 250 within the NST (Travers and Hu 2000). Although most neurons possibility is that they are interneurons with local connections a region responsive to avoided stimuli and hypothesized to be an area involved in oromotor reflexes like gaping (Halsell et al. 1993). Anatomical organization of neuron types

Fos immunohistochemistry indicated that NST neurons responsive to bitter stimuli and involved in the gape reflex are located in the medial third of the nucleus (reviewed in Travers and Travers 2005). Although this is somewhat consistent with the tendency for B-best neurons to be medially distributed, B-best neurons were not as far medial as the Fos data predicted, and instead overlapped with areas responsive to gustatory stimulation of the foliate and circumvallate in multunit recordings (Halsell et al. 1993). It is not clear whether this is indicative of different functions for the bitter-Fos and electrophysiologically identified neurons or simply explained by procedural variation because the Fos experiments used awake animals.

Central processing of bitter tastants

Recent molecular evidence suggests that the processing of aversive and appetitive tastants occurs in a separate but parallel fashion. Zuker and colleagues showed that T2Rs appear coexpressed in the same cells, whereas T1Rs, a family of receptors that binds amino acids and sweeteners, are not expressed in T2R-containing cells (Adler et al. 2000; Mueller et al. 2005; Nelson et al. 2001; Zhang et al. 2003). Our findings support the molecular data in that bitter responses arose predominantly from the posterior oral cavity where most T2Rs are located, and separate populations responded to bitter versus sweet stimuli or amino acids. In fact, although the unclustered B-best neurons tended to have less-specific profiles, CHX and DEN-best neurons were selective even with regard to other aversive stimuli (i.e., acids). These data are in contrast to the H/Q neurons described by Lemon and Smith (2005) that responded almost equivalently to salts, acids, and bitters. This discrepancy is likely the result of a combination of differences in receptive field stimulation, bitter stimuli chosen, and concentrations tested. The vast majority of B-best neurons in the present study had foliate RFs, a receptor population not stimulated in the previous study. In addition, test concentrations in the earlier experiment were chosen to achieve responses of equal magnitude in multunit recordings from the rostral pole of NST. This region is innervated mainly by the branches of the VIIth nerve (Hamilton and Norgren 1984), a nerve that responds much better to salts, acids, and sweeteners than bitter tastants (Frank et al. 1983; Nejad 1986; Sollars and Hill 2005). This procedure yielded test concentrations for salts and acids that were somewhat lower than those routinely used in neurophysiological studies; i.e., 10 mM NaCl and 3 mM HCl compared with 100 mM NaCl and 10 mM HCl (Lemon et al. 2003; Monroe and Di Lorenzo 1995; St John and Smith 2000). In fact, the NaCl concentration in the Lemon and Smith (2005) study (i.e., 10 mM) was just twice as high as the detection threshold of 5 mM reported by Geran and Spector (2000), whereas the QUI concentration (7 mM) was over two orders of magnitude above threshold (0.01 mM; St John and Spector 1996).
In the present study, the QUI concentration (3 mM) was somewhat lower than that commonly used in the CNS (7–20 mM; Di Lorenzo and Victor 2003; Jacobs et al. 1988; Lemon et al. 2003; Ogawa et al. 1988). However, it was one-half log step higher than that used by Frank (1991) to obtain vigorous responses in the glossopharyngeal nerve. Furthermore, this concentration is a potent behavioral stimulus because rats show almost complete avoidance of it in an unconditioned licking task (Geran et al. 2004; St John et al. 1994). In addition to activating B-best cells, both QUI and DEN produced sideband responses in a number of electrolyte-best neurons (i.e., A/N and N-cluster units) that railed the responses they produced in the B-best neurons, although they were just a fraction of the response to their own best stimulus. On the other hand, excitatory responses to the nonionic bitter tastants PROP and particularly CHX were more limited to B-best cells. Given that most A/N and N-cluster cells in this experiment had RFs in the AO, and most B-best cells received input from the foliates, this finding is consistent with accounts of ionic bitter stimulating the electrolyte-sensitive CT nerves of rodents, whereas nonionic bitter stimuli such as CHX and sucrose-octaacetate were largely ineffective (Damak et al. 2006; Danilova and Hellekant 2003; Frank et al. 2004; Kuwabara et al. 1970). This receptive field distinction for ionic versus nonionic bitter stimuli is also supported by the data reported by Lemon and Smith (2005). In their study most nonionic bitters, including CHX, resulted in poor multiunit responses in the rostral pole of NST, a region primarily innervated by the CT and GSP (Hamilton and Norgren 1984).

The selectivity of CHX and DEN cluster B-best neurons suggests the possibility that these cells possess the capacity to unambiguously signal the presence of bitter tastants. However, these B-best neurons were more narrowly tuned than many cells in the present sample and we do not necessarily propose other qualities are signaled in this fashion. In addition, the prototypical bitter stimulus QUI stimulated B-best neurons to some degree but was considerably less effective than the other bitter tastants, raising the possibility that selective B-best cells are associated only with certain bitter stimuli. Because narrowly tuned fibers robustly responsive to QUI are present in the glossopharyngeal nerve (Frank 1991), however, it seems possible that there is a similar population centrally, one that might become more apparent with a larger sample of B-best neurons and/or optimal stimulation of the circumvallate papilla.

The majority of B-best cells received input from the GL, a nerve thought to be more important for gustatory reflexes (Grill et al. 1992; Travers et al. 1987) than perception of taste quality (Markison et al. 1995; Spector and Grill 1992; Spector et al. 1997; St John and Spector 1998). Thus it is possible that neurons with highly selective responses to bitter stimuli serve mainly as the afferent limb for stereotyped behaviors like gaping and chin rubbing, which constitute protective rejection responses elicited preferentially by bitter tastants (Chan et al. 2004). Such a hypothesis is consistent with the tendency for B-best neurons and posterior mouth receptive fields to be less likely to project in the ascending gustatory pathway; i.e., to the PBN. Nevertheless, a role for B-best cells in perceptual aspects of quality coding cannot be ruled out because some of these selective cells did have AO receptive fields and/or projected to the PBN.

An across-neuron pattern mechanism that uses broadly tuned neurons to code “bitterness” likewise remains possible because ionic bitter tastants activate neurons with responses to multiple electrolytes (e.g., present data; Lemon and Smith 2005). However, to achieve acid versus bitter discrimination (Grove and Spector 2006; Myers and Sclafani 2003) or encode the taste quality of nonionic bitter stimuli, a pattern code would also have to incorporate neurons more selective for the bitter quality, or use additional mechanisms, such as some type of temporal coding (Di Lorenzo and Victor 2003; Katz et al. 2001).

Given the notable variety of neurons that respond to bitter tastants, it may be productive to consider labeled-line and pattern coding to constitute mechanisms that can operate in parallel, rather than viewing them as an “either-or” proposition. Perhaps the more broadly tuned neurons innervated by the VIIth nerve use an across-neuron pattern, whereas more narrowly tuned IXth nerve-innervated cells use a labeled line. These groups of neurons could operate independently to serve different functions, such as perceptual versus reflexive, but for certain behaviors could interact or be redundant (discussed in Spector and Travers 2005).

**Heterogeneity across bitter-best cells**

B-best neurons were usually activated by more than one bitter stimulus, but rarely by all four bitter tastants (Fig. 7), even though these stimuli produced an equivalent number of gaps (Chan et al. 2004; Supplemental figure). This was particularly noticeable for QUI, which produced a significant response in less than half of the cells (8/18 cells, Fig. 7). Further evidence of heterogeneity comes from the cluster analysis that produced two groups of B-best cells; one optimally responsive to CHX and a smaller group selective for DEN. That most neurons responded to more than one bitter stimulus is consistent with in situ hybridization demonstrating that individual TRCs express mRNAs for multiple T2Rs (Adler et al. 2000; Chandrashekar et al. 2000; Mueller et al. 2005). However, the molecular data also have been interpreted to suggest that each bitter-responsive TRC should respond similarly to all bitter stimuli, whereas our data indicate a certain amount of variation. This result is more indicative of differential distribution of bitter receptors across taste receptor cells, a hypothesis suggested by Ca2+/Ca2+ imaging and single-fiber recordings in the periphery (Caicedo and Roper 2001; Dahl et al. 1997). Together, these data suggest that individual T2R proteins could be expressed in varying levels across TRCs or that some bitter-responsive cells are capable of using a T2R-independent transduction mechanism. This latter hypothesis seems likely, given that some bitter stimuli, such as DEN, appear to activate TRCs directly without first binding to T2Rs under certain conditions (see Caicedo and Roper 2001; Caicedo et al. 2003; Chen and Herness 1997; Dotson et al. 2005; Peri et al. 2000; Rosenzweig et al. 1999; Sawano et al. 2005).

If this property proves unique to ionic bitter stimuli, it could help explain apparent differences between ionic and nonionic bitter representation in the brain stem.

This ionic versus nonionic distinction is also supported by the behavioral data. For instance, hamsters in a conditioned taste-aversion task cross-generalized between the ionic stimuli QUI and DEN, but failed to generalize a conditioned QUI aversion to the nonionic bitters sucrose octaacetate and caffeine (Frank et al. 2004). Similarly, Brassard et al. (2005) found that concentrations producing 50% lick suppression correlated highly for QUI and DEN across rats, less well for QUI and the...
nomonic CHX, and failed to reach significance for DEN and CHX. In fact, the ionic stimuli QUI and DEN appear so similar in taste quality that rats are unable to discriminate between these two compounds in an operant conditioning task (Spector and Kopka 2002). It will be important in the future to determine whether this lack of behavioral discrimination extends to other bitter tastants and whether the differential processing of bitter stimuli in the NST is preserved at higher levels.

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