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Gustatory Projections from the Nucleus of the Solitary Tract to the Parabrachial Nuclei in the Hamster

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Abstract

Taste-responsive cells in the nucleus of the solitary tract (NST) either project to the parabrachial nuclei (PbN) of the pons, through which taste information is transmitted to forebrain gustatory nuclei, or give rise to axons terminating locally within the medulla. Numerous anatomical studies clearly demonstrate a substantial projection from the rostral NST, where most taste-responsive cells are found, to the PbN. In contrast, previous electrophysiological studies in the rat have shown that only a small proportion (21–45%) of taste-responsive NST cells are antidromically activated from the PbN, suggesting that less than half the cells recorded from the NST are actually involved in forebrain processing of gustatory information. In the present experiment we investigated the projections from the NST to the PbN electrophysiologically in urethane anesthetized hamsters. Responses of 101 single neurons in the rostral NST were recorded extracellularly following lingual stimulation with 32 mM NaCl, sucrose and quinine hydrochloride (QHCl) and 3.2 mM citric acid. The taste-responsive region of the PbN was identified electrophysiologically and stimulated with a concentric bipolar electrode to antidromically activate each NST cell. Of the 101 taste-responsive NST cells, 81 (80.2%) were antidromically activated from the ipsilateral PbN. The mean firing rates to taste stimulation and the spontaneous activity of these projection neurons were significantly greater than those of non-projecting cells. Every sucrose-best neuron in the sample projected to the PbN. The mean conduction velocity of the 23 QHCl-best neurons was significantly lower than that of the other 58 PbN projection neurons, suggesting that the most QHCl-responsive cells are a subset of smaller neurons. These data show that a large majority of NST cells responsive to taste stimulation of the anterior tongue project to the gustatory subdivisions of the PbN and that these cells have the most robust responses to gustatory stimulation.

Introduction

The nucleus of the solitary tract (NST) and the parabrachial nuclei (PbN) are the first and second central relays in the rodent taste pathway, respectively (Norgren and Leonard, 1971; Norgren, 1978; Travers, 1988; Whitehead, 1990). Taste-responsive cells in the NST either project to the PbN to transfer taste information rostrally or give rise to axons terminating within the medulla (Norgren and Leonard, 1971; Norgren, 1978; Travers, 1988; Whitehead, 1990; Beckman and Whitehead, 1991; Halsell *et al.*, 1996). The NST consists of several subdivisions that differ in their afferent and efferent connections and their cytoarchitectural features (Davis and Jang, 1986; Travers, 1988; Whitehead, 1988, 1990; Halsell *et al.*, 1996). The rostral central and rostral lateral subnuclei in the NST receive input from the oropharyngeal cavity via the chorda tympani and greater superficial petrosal branches of the VIIth nerve and the lingual-tonsillar branch of the IXth nerve (Whitehead and Frank, 1983; Hamilton and Norgren, 1984; Brining and Smith, 1996). Most taste-responsive neurons are found in these two subnuclei (McPheeters *et al.*, 1990), from which neurons send axons to the pons to terminate largely in the caudal and ventral portions of the medial subdivision of the ipsilateral PbN (Herbert *et al.*, 1990; Whitehead, 1990). This area corresponds to the region of the PbN where taste-responsive cells have been recorded (Norgren and Pfaffmann, 1975; Van Buskirk and Smith, 1981). In contrast, neurons projecting to the reticular formation or the caudal NST have their origins mostly in the ventral and medial subdivisions of the rostral NST (Travers, 1988; Halsell *et al.*, 1996).

Whereas connections within the central taste pathway have been extensively explored anatomically, there are fewer physiological studies of these projections. Ogawa and colleagues (Ogawa *et al.*, 1980, 1984; Ogawa and Kaisaku, 1982) found that only 21% of NST neurons that responded to electrical stimulation of gustatory nerves and 31–34% of cells that responded to oral gustatory stimulation were antidromically activated by stimulation of the PbN in the

rat. A larger percentage (45%) was reported in an investigation by Monroe and Di Lorenzo (Monroe and Di Lorenzo, 1995), although it still comprised less than half of the taste-responsive neurons in their sample. In contrast to these electrophysiological results, Halsell *et al.* (Halsell *et al.*, 1996) reported that more cells in the rostral NST of the rat send axons to the PbN (67%) than to the reticular formation (33%). Thus the percentage of PbN projection cells in previous electrophysiological studies was likely underestimated. In these earlier experiments the PbN stimulating sites were determined solely on the basis of stereotaxic coordinates. In the present study we placed the stimulating electrode under electrophysiological guidance to ensure that it was positioned within the taste-responsive region of the PbN.

Materials and methods

Animals and surgery

Seventy-three male Syrian golden hamsters (*Mesocricetus auratus*) weighing 150–250 g were used in this experiment. Animals were deeply anesthetized with urethane (1.7 g/kg i.p.) and a cannula was inserted into the trachea to aid breathing. The animals were placed in a non-traumatic head holder with the head angled nose downward at 27° from the horizontal to minimize movement of the brainstem. The animal's body temperature was maintained at 37°C with a heating pad. The muscle over the occipital plate was cut along the midline and separated and a portion of the skull and dura was removed. The posterior portion of the cerebellum was aspirated to expose the floor of the IVth ventricle for 4–5 mm anterior to the obex, allowing direct access to the NST and PbN.

The concentric PbN stimulating/recording electrode was constructed by inserting an Epoxylite-insulated 33 gauge stainless steel tube into a 27 gauge stainless steel tube, the two being cemented together with Epoxylite 6001 (Epoxylite Corp., Irvine, CA). The inner tubing protruded ~500 μm from the outer barrel and was exposed at its tip for ~200 μm. The outer tubing was exposed concentrically for ~150 μm. A 75 μm diameter tungsten microelectrode (Frederick Haer & Co., Bowdoinham, ME) was inserted through the inner barrel of the stimulating electrode, its tip protruding 1 mm from the tip of the inner barrel. This combination stimulating/recording electrode was initially positioned ~4.0 mm rostral and 1.4 mm lateral to the obex to search for the taste-responsive region of the PbN (Van Buskirk and Smith, 1981). The microelectrode was lowered slowly into the pons to the depth at which the strongest neuronal activity was recorded in response to anodal current (50 μA, 0.5 s, 0.33 Hz) applied to the anterior tongue, which drives taste fibers of the chorda tympani (CT) nerve (Smith and Bealer, 1975). At that point the PbN electrode was lowered an additional 1 mm to position the stimulating electrode in the most taste-responsive area. It was then fixed to the adjacent skull with dental cement.

NST recording and taste stimulation

The taste responsiveness of a cell was initially determined by a change in neural activity associated with the application of anodal current pulses (50 μA, 0.5 s, 0.33 Hz) applied to the anterior tongue (Smith and Bealer, 1975). Action potentials were recorded from 101 taste-responsive NST cells with glass micropipettes (tip diameter 2 μm, resistance 7–10 MΩ) filled with a 2% (w/v) solution of Chicago Blue dye in 0.5 M sodium acetate. Cells responsive to lingual stimulation were encountered from 0.5 to 1.1 mm below the surface of the brain stem, with mean coordinates relative to the obex of 2.06 ± 0.09 mm anterior and 1.31 ± 0.09 mm lateral. Action potentials of single cells were isolated, displayed on oscilloscopes and monitored with an audio monitor. Extracellular potentials were differentially amplified (Bak MDA-41) and discriminated with a dual time–amplitude window discriminator (Bak DDIS-1). The amplified action potentials were counted online using a Pentium computer, configured with a CED 1401 *plus* interface board and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

A taste profile for each cell was established in response to four taste solutions, 32 mM sucrose, NaCl and quinine hydrochloride (QHCl) and 3.2 mM citric acid, applied to the anterior portion of the tongue. These concentrations evoke roughly equal multi-unit responses in the hamster NST (Duncan and Smith, 1992). The taste solutions were delivered by a gravity flow system composed of a two-way solenoid-operated valve connected via tubing to a distilled water rinse reservoir and a stimulus reservoir. The stimulation sequence, during which data were accumulated, was a continuous flow initiated by the delivery of 5 s of distilled water, followed by 10 s of stimulus and then by 5 s of distilled water. The flow rate was 2 ml/s. Following each taste stimulus, the tongue was rinsed with distilled water (50 ml) and individual stimulations were separated by at least 2 min to avoid adaptation (Smith and Bealer, 1976). Each cell was categorized as responding best to sucrose, NaCl, citric acid or QHCl on the basis of its response profile (see Frank, 1973).

Classification of PbN projection and non-projection neurons

To test each NST cell for antidromic invasion, rectangular pulses (0.5 ms, <120 μA) were delivered to the taste-responsive area of the PbN. Three criteria defined antidromic activation (Iggo, 1958). First, the action potentials of a projection neuron should be evoked at a constant latency. Second, the projection neuron should be able to follow paired pulse stimulation at >200 Hz. Finally, a collision test was conducted between spontaneous and stimulus-evoked action potentials. If a taste-responsive NST cell failed to

meet any one of these criteria, it was classified as a non-projection neuron. For each PbN projection neuron the threshold of PbN stimulation was defined as the lowest stimulus intensity that would produce an antidromic action potential on five consecutive trials. Both antidromic threshold and latency were measured for each cell.

At the end of each experiment the recording site in the NST was marked with Chicago Blue dye by passing a 10 μA cathodal current through the recording electrode for 10 min. The stimulating site in the PbN was evident from tissue damage produced by the stimulating electrode. The linear distance between the stimulating site in the PbN and recording site in the NST was measured to estimate the conduction velocity of each PbN projection neuron. The hamster was then given an overdose of urethane and perfused through the heart with 4% formaldehyde containing 3% potassium ferrocyanide and ferricyanide. Following removal of the brain, 40 μm frozen sections were cut in the coronal plane and stained with Neutral Red.

Data analysis

Responses to taste stimuli were quantified by subtracting the 5 s pre-stimulus baseline from the first 5 s of the evoked response to yield a net response (impulses/5 s). Responses are reported as means ± SEM. Differences in mean firing rates between projection and non-projection neurons, among taste stimuli and in conduction velocities were compared using ANOVA.

Results

Histology

The recording and stimulating sites were examined histologically. A recording site in the NST is shown in Figure 1A. This cell was a QHCl-best PbN projection neuron, located medial to the solitary tract, most likely in the rostral central subdivision. Most recording sites were found near the level of the NST where the dorsal cochlear nucleus (DC) is first apparent on the dorsolateral margins of the medulla. This region of the NST receives its predominant gustatory

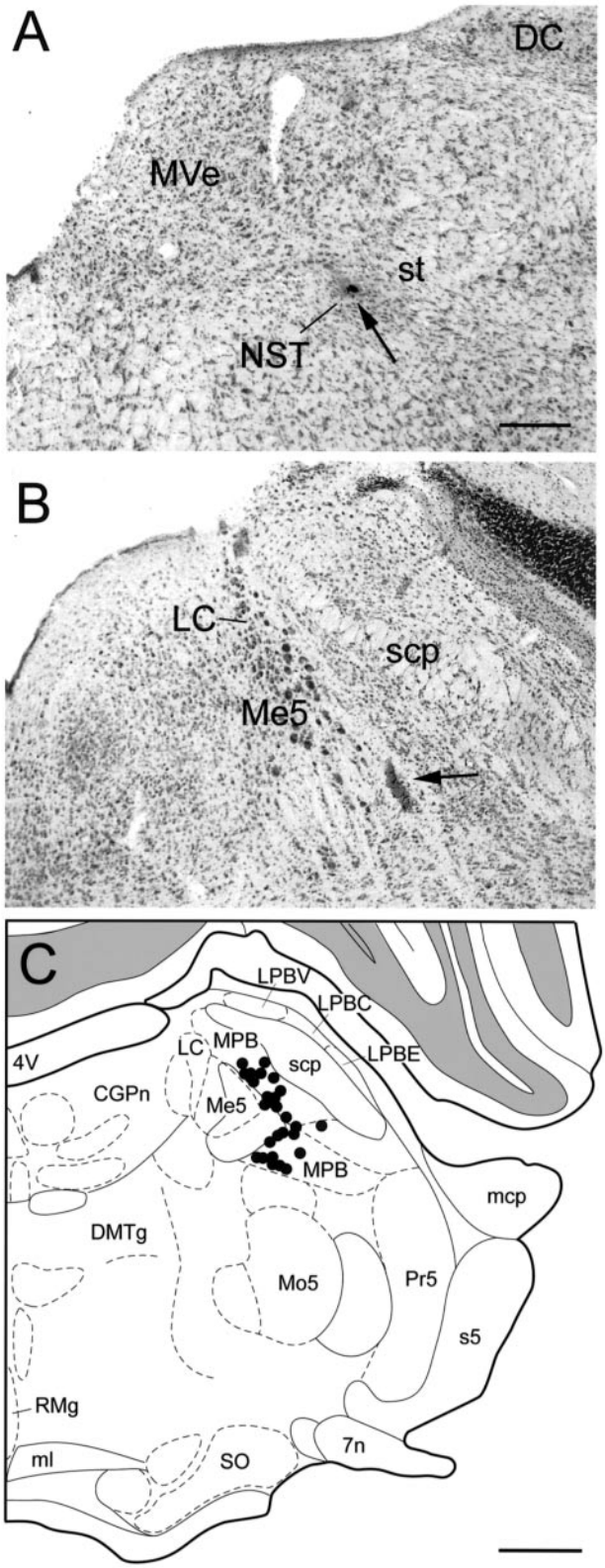

Figure 1 Photomicrographs of Neutral Red stained sections showing the locations of stimulating and recording electrodes. **(A)** Coronal section through the medulla showing a recording site in the NST. This section was at the same rostrocaudal plane as the caudal portion of the dorsal cochlear nucleus (DC). The recorded cell (arrow) was located medial to the solitary tract, most likely in the rostral central subdivision of the NST. **(B)** Coronal section through the caudal pons showing a stimulating site in the PbN. The electrode tip projected to the area just ventral to the superior cerebellar peduncle (arrow). **(C)** Positions of 28 PbN stimulating sites reconstructed onto a standard atlas section through the hamster PbN. 4V, fourth ventricle; 7n, facial nerve; CGPn, central gray of pons; DC, dorsal cochlear nucleus; DMTg, dorsomedial tegmental area; LC, locus coeruleus; LPBC, LPBE, LPBV, lateral parabrachial nucleus, central, external and ventral; mcp, middle cerebellar peduncle; Me5, mesencephalic trigeminal nucleus; ml, medial lemniscus; Mo5, motor trigeminal nucleus; MPB, medial parabrachial nucleus; MVe, medial vestibular nucleus; NST, nucleus of the solitary tract; Pr5, principal sensory trigeminal nucleus; RMg, raphe magnus nucleus; s5, sensory root of trigeminal nerve; scp, superior cerebellar peduncle; SO, superior olive; st, solitary tract. Calibration bar: (A, B) 300 μm; (C) 500 μm. Medial is to the left in all panels.

input from the VIIth nerve (Whitehead and Frank, 1983; Whitehead, 1988). We were unable to unambiguously assign each recorded cell to a nuclear subdivision, although all of the recorded cells appeared to be in the region of the NST corresponding to the rostral central or rostral lateral subdivisions, where the majority of PbN projecting neurons have been localized anatomically (Whitehead, 1990).

An example of the tissue damage induced by the stimulating/recording electrode in the PbN is shown in Figure 1B. The electrode was positioned ventral to the superior cerebellar peduncle (scp) within the medial PbN, fairly caudal within the parabrachial nuclear complex. This area was highly responsive to anodal stimulation of the tongue (see Materials and methods) and has previously been shown to contain taste-responsive neurons (Van Buskirk and Smith, 1981; Halsell and Frank, 1991) and to receive the majority of axons from the taste-responsive portions of the NST (Whitehead, 1990). The mean coordinates of the stimulating sites were 4.08 ± 0.12 mm anterior to the obex and 1.48 ± 0.11 mm lateral to the midline. The positions of 28 of the stimulating electrodes are depicted in Figure 1C on a standard drawing of the hamster brain (Morin and Wood, 2001), showing them to be distributed within the medial parabrachial nucleus (MPB). Although not all of the sites were histologically reconstructed, every stimulating electrode was placed in a taste-responsive site in the PbN (see Materials and methods).

Ascending projection from the NST to the gustatory region of the PbN

A total of 101 taste-responsive cells were recorded extracellularly from the NST of 73 hamsters. To determine the projection status of an NST cell, we tested whether it was antidromically invaded from the gustatory portion of the ipsilateral PbN. Electrical stimulation of the PbN gustatory area produced one of three effects in NST taste-responsive neurons: each cell discharged a spike either antidromically or orthodromically or showed no response to PbN stimulation.

Among these 101 NST neurons, 81 (80.2%) were driven antidromically by ipsilateral PbN stimulation and classified as PbN projection neurons. These projection neurons showed constant latencies to PbN stimulation. We measured the threshold of the PbN stimulation required to evoke an antidromic response and the antidromic latency for every PbN projection cell. The mean (± SEM) antidromic threshold was 48.33 ± 5.54 μA and the mean latency was 4.14 ± 0.41 ms. An electrical stimulus of 50 μA would be expected to affect an area ~0.5 mm in diameter (Ranck, 1975). Each NST cell produced action potentials with a constant latency following paired pulse stimulation of the PbN at high frequency (>200 Hz at 1.2× threshold). Collision tests were conducted at the same intensity as the paired pulse stimulation for all 81 PbN projection cells, as all of these neurons were spontaneously active. The lowest spontaneous rate was 0.2 impulses/s, which occurred in two cells. In the collision test the first evoked action potential of each pair was cancelled as it met the spontaneously generated action potential, which was used to trigger the paired pulse stimulation. Figure 2 demonstrates the fulfillment of criteria for antidromic invasion from the ipsilateral gustatory PbN in two NST neurons.

Taste-responsive NST cells that did not show antidromically evoked action potentials following PbN stimulation were categorized as non-projection neurons. Of the 20 non-projection neurons, action potentials were evoked orthodromically in six cells, as evidenced by variable response latencies indicative of synaptic transmission. The variances in the latency of action potentials of three non-projection cells are shown in Figure 3. The other 14 neurons did not respond to PbN stimulation.

Conduction velocity of PbN projection neurons

The conduction velocity of each PbN projection neuron was estimated by dividing the distance between the PbN stimulating and NST recording sites by the latency of the antidromic response. The frequency distribution of conduction velocities was essentially bimodal (Figure 4). Although all of these cells were relatively slowly conducting, most displayed conduction velocities >0.6 m/s. However, there was a substantial subset of neurons (20/81, 24.7%) with much slower conduction velocities (<0.3 m/s), all of which responded best to QHCl. The 23 QHCl-best neurons each had a conduction velocity <0.5 m/s. The mean conduction velocity of all 23 QHCl-best neurons was 0.25 ± 0.02 m/s, whereas that of the other 58 PbN projection cells was 0.95 ± 0.04 m/s. The difference between the QHCl-best neurons and all of the other groups was significant [$F(3,77) = 41.66$, $P < 0.001$; Bonferroni *post hoc* test, $P < 0.05$]. This result suggests that QHCl-best neurons are smaller and/or have axons of smaller diameter than other cell types.

Taste response characteristics of PbN projection versus non-projection neurons

Each of the 101 NST neurons was tested for its responsiveness to the four taste stimuli and categorized as sucrose-, NaCl-, citric acid- or QHCl-best on the basis of its response profile. Among 81 PbN projection neurons, NaCl-best neurons ($n = 35$) comprised the largest category. There were 23 QHCl-best neurons, 14 sucrose-best neurons and nine citric acid-best cells in the PbN projection group. Figure 5 shows gustatory responses of four PbN projection neurons, one from each best stimulus category. In comparison, among the non-projection neurons there were no sucrose-best, eight NaCl-best, seven citric acid-best and five QHCl-best cells.

Comparison of the response profiles revealed differences between PbN projection and non-projection neurons. Mean (± SEM) firing rates to taste stimulation and the spontaneous activity of 81 PbN projection neurons were 32.9 ±

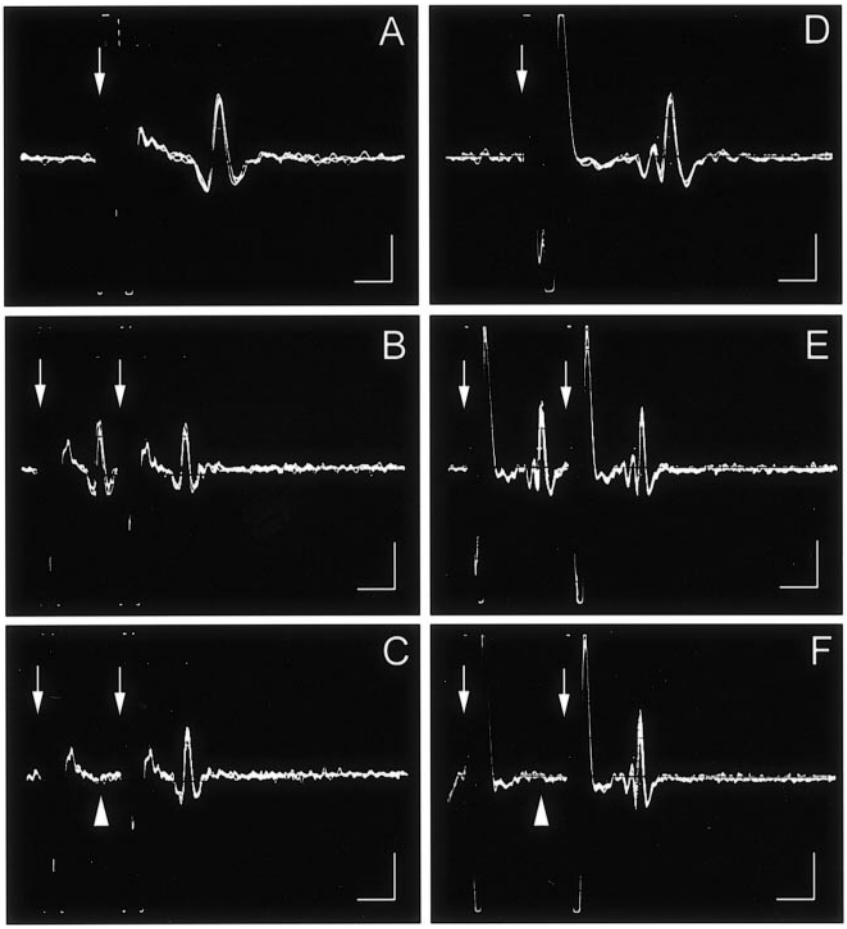

Figure 2 Antidromically activated action potentials recorded from two different taste-responsive cells in the NST **(A–C, D–F)**. Each record is composed of a minimum of three superimposed oscilloscope traces. Antidromic spikes of constant latency were evoked from each cell (A, D). Each neuron was able to follow high frequency (e.g. 200 Hz) antidromic stimulation of the PbN (B, E). Collision of spontaneous and antidromic spikes caused elimination of the antidromic spike, which should have occurred at the white arrowhead (C, F). Calibration bars: (A, D) 1 ms; (B, C, E, F) 2 ms; 1 mV. PbN stimulation was delivered at the arrows.

2.6 and 19.0 ± 2.2 impulses/5 s, respectively. In contrast, those values in the 20 non-projection neurons were 16.1 ± 2.2 and 7.7 ± 1.7 impulses/5 s. All responses, including spontaneous rate, were significantly greater in PbN projection than non-projection cells [$F(1,495) = 13.59$, $P < 0.001$]. Not only were all sucrose-best cells found in the PbN projection group, but sucrose showed a larger percentage difference between the PbN projection and non-projection cells (72.1%) than the other three stimuli (NaCl, 57.9%; citric acid, 23.0%; QHCl, 48.5%). Figure 6 shows the mean response rates to each taste stimulus and spontaneous rates in the PbN projection and non-projection neurons. Although concentrations of taste stimuli were chosen that evoke roughly equal multi-unit responses in the NST, the mean responses to the four stimuli differed slightly from one another [$F(3,396) = 2.69$, $P < 0.05$) due to the greater numbers of NaCl-best neurons (Bonferroni *post hoc* test, $P < 0.05$). The taste responses of all 101 NST cells are shown in Figure 7, where the differences in firing rate between projection and non-projection cells are readily apparent.

Discussion

Projection status of NST taste-responsive neurons

This study provides evidence that a large majority (80.2%) of taste-responsive neurons in the hamster NST project to the gustatory area of the ipsilateral PbN. Numerous anatomical studies show that the next synapse beyond the NST in the ascending taste pathway of rodents occurs in the PbN (Contreras *et al.*, 1982; Hamilton and Norgren, 1984). Although Halsell *et al.* (Halsell *et al.*, 1996) demonstrated that more cells in the rostral NST project to the PbN than to the reticular formation or the caudal NST in rats, it is impossible in such anatomical studies to know whether these cells are gustatory in function. Indeed, previous electrophysiological studies have reported that considerably fewer than half of the gustatory neurons in the NST are antidromically activated by stimulation of the PbN (Ogawa *et al.*, 1980, 1984; Ogawa and Kaisaku, 1982; Monroe and Di Lorenzo, 1995).

Either the percentage of NST cells projecting to the PbN

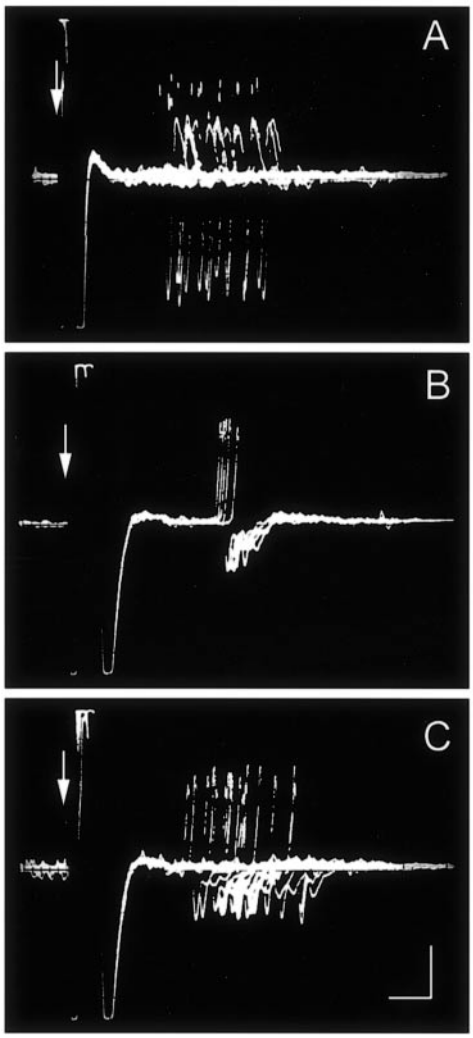

Figure 3 **(A–C)** Orthodromic spikes in three NST cells are shown. PbN stimulation (arrows) evoked action potentials of varying latency in these cells. Calibration bars: (A) 2 ms; (B, C) 1 ms; 1 mV.

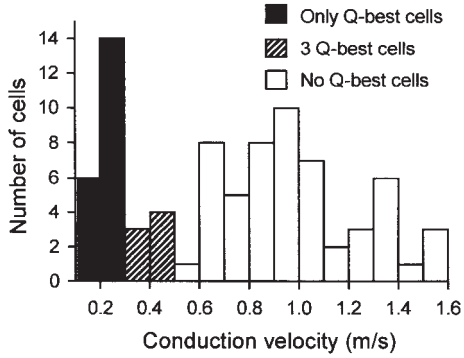

Figure 4 Frequency distribution of the conduction velocities (m/s) of the 81 NST neurons that were antidromically activated by PbN stimulation (PbN projection neurons). Most cells had conduction velocities >0.6 m/s (open bars). However, there was a subset of neurons (solid bars) with much slower conduction velocities (<0.3 m/s). The 23 QHCL-best neurons all had conduction velocities <0.5 m/s.

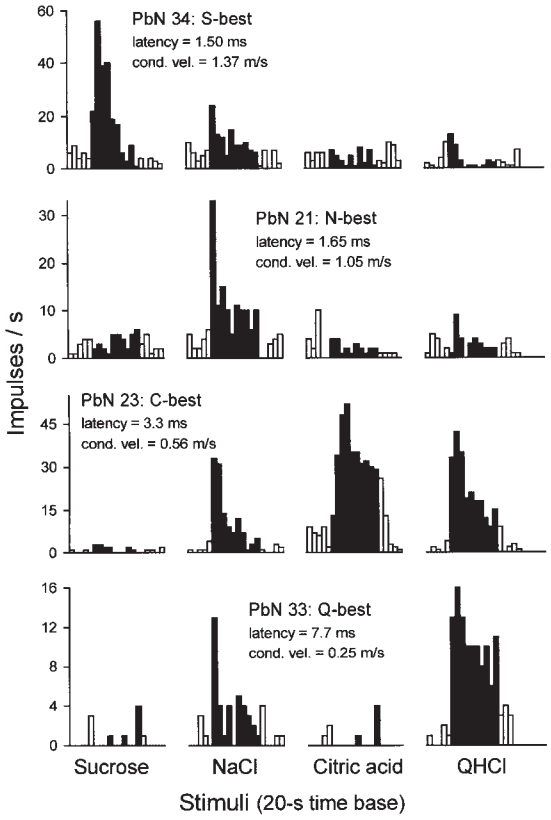

Figure 5 Taste response profiles of four antidromically activated cells in the NST. The 10 s responses to each of the four taste stimuli are shown for each cell (black bars), preceded and followed by 5 s of distilled water (open bars). One cell is shown from each of the best stimulus categories (sucrose-, NaCl-, citric acid- and QHCl-best).

in these previous electrophysiological experiments was underestimated or many of the neurons shown to project to the PbN in anatomical studies are non-gustatory in function. In these earlier electrophysiological investigations the PbN stimulating electrode was positioned stereotaxically. Monroe and Di Lorenzo (Monroe and Di Lorenzo, 1995) used only those placements determined to be within the PbN taste area on histological examination for their analysis. These investigators reported that 45% of the NST neurons were PbN projection cells, compared with the 21–34% reported in earlier studies (Ogawa *et al.*, 1980, 1984; Ogawa and Kaisaku, 1982). However, even such histological confirmation cannot eliminate the possibility that the stimulating electrode was positioned in a non-gustatory region of the PbN. In the present study our combination stimulating/recording electrode made it possible to place the stimulating electrode precisely at a taste-responsive location within the PbN. This approach resulted in 80.2% of the neurons being antidromically activated by PbN stimulation. Therefore, the larger differences reported here between these two categories of cells than in previous studies probably reflects the inclusion of many PbN projection neurons in the non-projection group in the earlier investigations. In the present study PbN

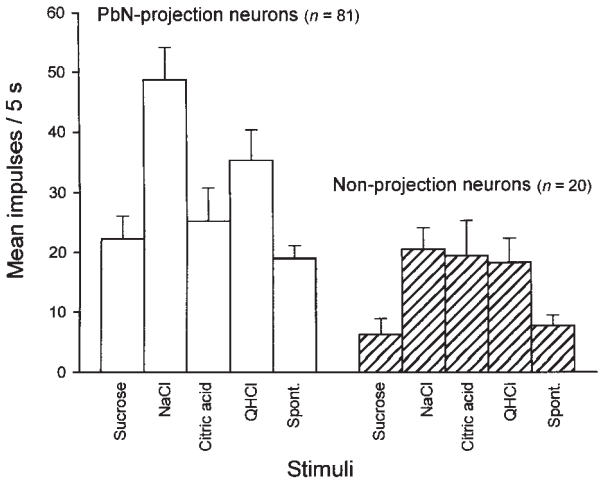

Figure 6 Mean (± SEM) firing rate of PbN projection and non-projection neurons of the NST. Responses to the four taste stimuli are net responses (above spontaneous rate); responses for spontaneous rate show the average across all cells in that category.

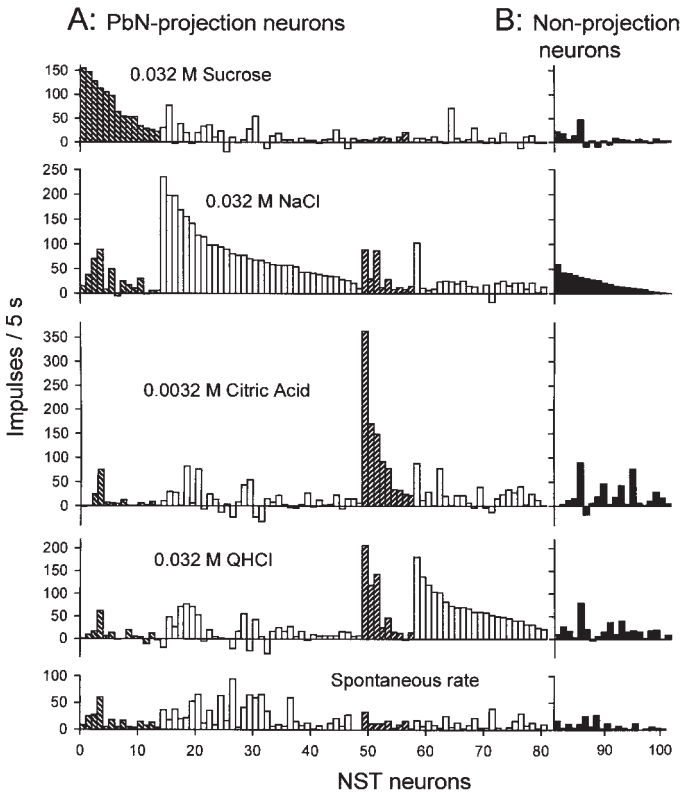

Figure 7 Net responses (impulses/5 s above or below spontaneous rate) of 101 NST neurons to sucrose, NaCl, citric acid and QHCl. **(A)** Responses of 81 PbN projection neurons; neurons are arranged along the abscissa according to their best stimulus, with neurons 1–14 being sucrose-best (shaded), 15–49 NaCl-best (unfilled), 50–58 citric acid-best (shaded) and 59–81 QHCl-best (unfilled). Cells are arranged within each best stimulus group according to the magnitude of the response to their best stimulus. **(B)** Responses of 20 non-projection neurons; cells are arranged in order of their response to NaCl. The response profile of any one cell can be read from top to bottom. The spontaneous rate of each cell (during distilled water) is shown at the bottom of the figure.

projection neurons were significantly more numerous and more responsive than non-projection cells. Although it is possible that species difference could explain the discrepancies between the present hamster experiment and the previous rat work, anatomical studies have shown no remarkable differences between rats and hamsters in the size or distribution of the projection from the NST to the PbN (Contreras *et al*., 1982; Whitehead and Frank, 1983; Hamilton and Norgren, 1984; Whitehead, 1990).

The proportion of projection neurons could also be related to the distribution of cells recorded from the NST. In earlier studies the recorded neurons, regardless of their projection status, were distributed across the entire mediolateral extent of the NST (Ogawa *et al*., 1984) or confined to the lateral half of the rostral NST (Monroe and Di Lorenzo, 1995). In both series of studies in the rat the PbN projection neurons were intermingled with those that were not antidromically activated by PbN stimulation. In addition, the entire oral cavity was stimulated in these earlier studies, whereas stimuli were applied only to the anterior tongue in the present experiment. Thus the proportion of PbN projection neurons in the present study reflects NST cells activated by VIIth nerve input. Because the subdivisions of the hamster NST (Whitehead, 1988) are difficult to visualize in this counterstained material, we were not confident in determining the cytoarchitectural boundaries of the NST subdivisions with sufficient precision to relate the positions of the cells to these landmarks. However, the recorded cells were located centrally within the NST, medial to the solitary tract, in a region that likely corresponds to either the rostral central or rostral lateral subdivision (see Figure 1A), where most PbN projecting neurons have been located anatomically (Whitehead, 1990).

The location of the sites of PbN stimulation corresponded quite closely to the area from which taste responses to anterior tongue stimulation have been recorded from the hamster PbN (Van Buskirk and Smith, 1981; Halsell and Frank, 1991). The mean coordinate of the PbN stimulation sites was 4.08 ± 0.12 mm anterior to the obex, which is relatively caudal in the PbN. In hamsters many taste-responsive neurons are found ventral to the brachium conjunctivum (see Figure 1C) at this level [cf. figure 1A and B in (Van Buskirk and Smith, 1981)] and axons from the taste-responsive regions of the NST terminate most heavily in this part of the pons (Whitehead, 1990). We expect that even NST neurons that project to more rostral levels of the PbN would be stimulated in these experiments, as their axons must pass the stimulating electrode en route to the rostral PbN.

Taste responsiveness of PbN projection versus non-projection neurons

The present results show that PbN projection neurons

respond more vigorously to taste stimuli than NST neurons that do not send axons to the PbN (see Figures 6 and 7). This difference was greatest for the response to sucrose and least for the response to citric acid. There were no sucrose-best neurons that did not project to the PbN. Ogawa *et al.* (Ogawa *et al.*, 1984) found fewer sucrose-best cells in the non-projection group than in the PbN projection group and the average response to sucrose in the non-projection cells was less than half that of the PbN projection neurons. In the investigation by Monroe and Di Lorenzo (Monroe and Di Lorenzo, 1995) the response to sucrose in the PbN projection neurons was also greater than in non-projection neurons. All of these studies suggest that information about the taste of sucrose is preferentially directed toward forebrain gustatory areas.

In contrast to the responsiveness to sucrose, previous studies have reported that the response to QHCl was not significantly different between PbN projection and non-projection cells and that only a few QHCl-best cells were found in the PbN projection category (Ogawa *et al.*, 1984; Monroe and Di Lorenzo, 1995). These differences in the responsiveness of NST neurons to sucrose and QHCl led these previous investigators to suggest that the responses of PbN neurons serve to enhance the differences between palatable and unpalatable taste stimuli. However, our findings do not support this hypothesis. First, the mean response of PbN projection neurons to QHCl was almost twice that of non-projection cells (35.51 versus 18.25 impulses/5 s). Second, we found many QHCl-best cells among the PbN projection neurons: more than the number of sucrose-best neurons and more than the number of QHCl-best cells that do not project to the PbN. This result indicates that information about unpalatable tastes is readily transferred to the gustatory PbN from the NST.

QHCl has been used as an exemplary bitter taste stimulus in behavioral and electrophysiological studies and evokes avoidance behavior in rodents even at very low concentrations (Grill and Norgren, 1978). The PbN plays an important role in conditioned taste aversion, which is a critically important learning mechanism that prevents the repeated ingestion of toxic food (Kiefer, 1985). The PbN must be intact for the retention of a conditioned taste aversion (Grigson *et al.*, 1997). In addition, Yamamoto and Sawa (Yamamoto and Sawa, 2000) have demonstrated a population of cells within the rat PbN that show Fos activation following lingual application of QHCl. Electrophysiological evidence from awake rats also suggests that PbN neurons can differentiate QHCl from the other stimuli (Nishijo and Norgren, 1997). Thus the transfer of information about unpalatable stimuli to the pons clearly occurs and may be necessary for ingestive decisions based on prior gustatory experience.

Conduction velocity of QHCl-best neurons

We found many more QHCl-best neurons in the present investigation than previous electrophysiological studies have reported; there are several factors that might account for this discrepancy. We used glass micropipettes (7–10 MΩ) to record from cells in the NST, which would facilitate the isolation of relatively small cells. In previous electrophysiological studies in our laboratory using these electrodes we have found QHCl-best neurons to be a considerable portion of taste-responsive NST cells in hamsters when this relatively high concentration (32 mM) of QHCl was used (Li and Smith, 1997; Smith and Li, 1998, 2000). The concentration of QHCl in the present study was relatively higher than in many previous electrophysiological investigations (cf. Van Buskirk and Smith, 1981); a lower QHCl concentration would have resulted in many of these QHCl-best cells being classified as citric acid-best (see Figure 7) or as NaCl-best (see PbN 33 in Figure 5). However, cells classified as QHCl-best by this stronger QHCl concentration displayed a distinctive feature: they all had very slow conduction velocities, i.e. even though the classification of these cells by their best stimulus depends strongly on the stimulus concentrations used (Smith and Travers, 1979), this strong QHCl concentration identified a physiologically unique group of neurons.

Since conduction velocity is directly related to cell or fiber size (Webber and Pleschka, 1976; Harper and Lawson, 1985), we conclude that QHCl-best neurons are likely to be considerably smaller than other cell types. In addition to classifying gustatory cells according to their physiological properties, there have been attempts to categorize them based on morphological features (Davis and Jang, 1986, 1988; Lasiter and Kachele, 1988; Whitehead, 1990; Renehan *et al.*, 1994, 1996; Leonard *et al.*, 1999). However, it has been difficult to relate these two classifications to one another. For example, there is no clear relationship between morphological and functional features of rat NST neurons, except that those cells responding very narrowly to QHCl have been shown to be significantly smaller than other neurons (Renehan *et al.*, 1996). Even though these data could not account for all QHCl-best neurons in the NST, they implied that QHCl-sensitive neurons might be morphologically unique. The present finding of slow conduction velocities of QHCl-best neurons supports this hypothesis.

Orthodromic activation

The 20 neurons that were not antidromically activated by PbN stimulation could be interneurons or neurons that project to the reticular formation or motor nuclei in the medulla (Travers and Norgren, 1983). It is also possible that some of these neurons project to the contralateral PbN, although that possibility was not tested in the present investigation. A bilateral projection from the rostral NST to the medial PbN has been demonstrated anatomically in rats (Williams *et al.*, 1996). Whereas the primary role of NST neurons that project to the PbN is most likely to convey taste information to higher gustatory nuclei, non-projection

neurons probably play a role in brainstem circuits or as interneurons within the NST. About 18% of neurons in the hamster NST are small ovoid cells expressing GABA-like immunoreactivity (Davis, 1993). These stand in contrast to the numerous elongate and stellate neurons that project axons to the PbN (Whitehead, 1990). Thus the 20% of taste-responsive cells that were not antidromically activated by PbN stimulation in the present study likely correspond to these small GABAergic interneurons.

We found six taste-responsive cells in the NST that were orthodromically driven from the ipsilateral PbN. Karimnamazi and Travers (Karimnamazi and Travers, 1998) showed that neurons in the taste-responsive region in the waist area of the PbN project caudally to the lateral parvocellular reticular formation in the medulla; some of the descending fibers appeared to terminate within the rostral NST. These combined results imply that taste information in the NST could be modulated by descending input from the PbN. Taste-responsive cells in the NST are under the influence of corticofugal projections in the rat (Di Lorenzo and Monroe, 1995) and hamster (Smith and Li, 2000). Descending modulation of NST cells by the lateral hypothalamus and the central nucleus of the amygdala has also been shown in the rat (Bereiter *et al.*, 1980; Matsuo *et al.*, 1984; Murzi *et al.*, 1986) and hamster (Cho *et al.*, 2000; Li *et al.*, 2000). Whether descending fibers from the PbN alter taste function in NST cells has not been investigated.

In conclusion, we have demonstrated that the vast majority of taste-responsive NST cells transfer taste information, including information about both palatable and unpalatable tastes, to the gustatory PbN. We also found that QHCl-best neurons appear to be relatively smaller in size than other cell types, based on their slow antidromic conduction velocities. In contrast to previous electrophysiological investigations in the rat, the present results confirm that most taste-responsive cells of the NST participate in the projection of taste information to the forebrain.

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