Modulation of activity of gustatory neurons in the hamster parabrachial nuclei by electrical stimulation of the ventroposteromedial nucleus of the thalamus

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Mao L, Cho YK, Li C-S. Modulation of activity of gustatory neurons in the hamster parabrachial nuclei by electrical stimulation of the ventroposteromedial nucleus of the thalamus. Am J Physiol Regul Integr Comp Physiol 294: R1461–R1473, 2008. First published March 5, 2008; doi:10.1152/ajpregu.00802.2007.—The parvicellular part of the ventroposteromedial nucleus of the thalamus (VPMpc) is positioned at the key site between the gustatory parabrachial nuclei (PbN) and the gustatory cortex for relaying and processing gustatory information via the thalamocortical pathway. Although neuroanatomical and electrophysiological studies have provided information regarding the gustatory projection from PbN to VPMpc, the exact relationship between PbN and VPMpc, especially the efferent projection involving VPMpc to PbN, is obscure. Here we investigated the reciprocal connection between these two gustatory relays in urethane-anesthetized hamsters. We recorded from 114 taste-responsive neurons in the PbN and examined their responsiveness to electrical stimulation of the VPMpc bilaterally. Stimulation of either or both of the ipsilateral or contralateral VPMpc antidromically activated 109 gustatory PbN neurons. Seventy-two PbN neurons were antidromically activated after stimulation of both sides of the VPMpc, indicating that taste neurons in the PbN project heavily to the bilateral VPMpc. Stimulation of VPMpc also orthodromically activated 110 of PbN neurons, including 106 VPMpc projection neurons. Seventy-eight neurons were orthodromically activated bilaterally. Among orthodromic activations of the PbN cells, the inhibitory response was the dominant response; 106 cells were inhibited, including 10 neurons that were also excited contralaterally, indicating that taste neurons in the PbN are subject to strong inhibitory control from VPMpc. Moreover, stimulation of VPMpc altered taste responses of the neurons in the PbN, indicating that VPMpc modulates taste responses of PbN neurons. These results may provide functional insight of neural circuitry for taste processing and modulation involving these two nuclei.

central gustatory pathway; pons; electrophysiology

THE PARABRACHIAL NUCLEI (PbN) and the parvicellular part of the ventroposteromedial nucleus of the thalamus (VPMpc) are the second and tertiary central relays for taste information processing in rodents, respectively (4, 13, 17, 28, 45, 49, 51). Gustatory information elicited from the tongue and oropharyngeal cavity is initially carried to the rostral portion of the nucleus of the solitary tract (NST) by the VIIth, IXth, and Xth cranial nerves, and these terminals are distributed in a topographic fashion with rostral-caudal sequence in the rostral NST (2, 9, 23, 24, 89). From the NST, ascending gustatory fibers project to third-order taste cells within the PbN of the pons (7, 22, 42, 46, 53–55, 87). Two separate routes carry taste information from the PbN further to the forebrain taste areas and cortex: the ventral forebrain pathway that involves the central nucleus of the amygdala (CeA), the lateral hypothalamus (LH), the bed nucleus of the stria terminalis (BNST), the substantia innominata (3, 12, 16, 21, 31, 33, 34, 41, 47, 63, 64, 71); and the thalamocortical pathway that is characterized by the involvement of the bilateral VPMpc, which, in turn, carries taste information to the gustatory cortex (GC) (5, 25, 45, 48, 51).

The PbN is a critical neural substrate for a number of taste-guided behavioral or physiological responses, including conditioned taste aversion learning (CTA), gastric distension, and sodium appetite. It has been demonstrated that a subpopulation of neurons in the PbN can be coactivated by both gustatory and vagal (hepatic) stimulation (26) or gustatory and gastric distension (1), indicating that the PbN is the locus of integration for gustatory and other vagal afferent inputs. It is also known that physiological factors associated with ingestive behavior modulate taste responses of the PbN neurons. For instance, intraduodenal lipid infusion decreases the response magnitude of taste neurons in the PbN with the largest reduction in sucrose responses in awake rats (20). Gustatory responses of PbN neurons to NaCl in sodium-deprived rats were reduced relative to the control animals (70). Lesions of PbN also block the expression of a sodium appetite in rats (14, 66), suggesting that PbN is involved in mediating sodium appetite. In addition, studies have demonstrated that lesions of the PbN prevent acquisition of CTA in rats (19, 59, 69, 77, 90).

A number of anatomical and electrophysiological studies have shown strong neuronal connections between the PbN and forebrain gustatory nuclei. The efferent projections to the gustatory PbN from the LH, CeA, BNST, and GC were demonstrated in rats and hamsters (41, 83, 84, 88, 94). These forebrain nuclei likely have a role in gustatory modulation of the cells in the PbN. Indeed, electrophysiological investigations have reported that gustatory responses of pontine neurons are modulated by electrical stimulation of the LH, CeA, and GC in the hamster and the rat (11, 35, 39, 40).

The VPMpc, the third-order gustatory relay in the rodent, is less understood in several aspects (56, 68, 85, 86). For example, whereas the afferent projections from the PbN to the VPMpc were well documented, the efferent projections from the VPMpc to the PbN were not demonstrated (4, 21, 64). In

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addition, while earlier studies have shown that the VPMpc is involved in taste detection and discrimination (32), sodium appetite (30, 44), and CTA (37, 92, 93), more recent studies have demonstrated that the VPMpc has a minimal influence on innate taste preference (15, 62, 78), sodium appetite (14, 65), or CTA (14, 65). Thorough reviews of earlier studies suggested that the claim of the involvement of VPMpc in taste preference, sodium appetite, and CTA were derived from the lesions that were misplaced, inordinately large, or destroyed fibers of passage (58, 60, 69). More recent investigations indicated that the VPMpc is instead involved in more complex gustatory information processing tasks, e.g., anticipatory contrast effect in which rats reduce intake of an otherwise palatable (saccharin) solution when presentation of this solution predicts access to a more preferred (sucrose) solution over repeated daily pairings (18, 61, 67).

The involvement of the forebrain gustatory nuclei in the ventral forebrain pathway, e.g., LH, CeA, BNST, and GC, on modulation of activity of PbN taste neurons has been well documented (10, 11, 34, 35, 39, 40). However, the possibility of the involvement of the VPMpc in the thalamocortical pathway on modulation of activity of taste neurons in the PbN has never been investigated. Earlier electrophysiological studies concerning parabrachio-thalamic relay were focused mainly on the projection status of PbN taste neurons to the VPMpc but not on neuronal modulation of gustatory PbN neurons (25, 45, 47, 51, 52). The purpose of the present study was to examine the effect of electrical stimulation of the VPMpc on the responses of the gustatory PbN neurons and whether VPMpc stimulation modulates gustatory responses of the neurons in the hamster PbN.

MATERIALS AND METHODS

Animal and surgery. All procedures in the animal experiments were in accordance with the guidelines on the use and care of laboratory animals set by National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Southern Illinois University at Carbondale. Young, adult male Syrian golden hamsters (Mesocricetus auratus, Harlan Sprague Dawley, Indianapolis, IN), weighing between 130 and 185 g (n = 34), were deeply anesthetized with urethane (1.7 g/kg ip). An additional anesthetic (10% of original dose) was given as needed during the course of each experiment to maintain anesthesia.

Each animal was tracheotomized and mounted in a stereotaxic instrument (Narishige SR-6N) using an auxiliary ear bar (EB-4), with the incisor bar at the same level as the interaural line. The tissue overlying the parietal bone was removed, and a hole was drilled on each side of the skull to access the VPMpc. A concentric bipolar stimulating electrode, constructed from 26-gauge stainless steel tubing and 140-μm-thick stainless steel wire, was lowered into the VPMpc on each side of the brain (coordinates: 1.1 mm posterior to the bregma, 0.9 mm lateral to the midline, and 5.3 mm ventral to the brain surface) and secured with dental cement. The electrodes, except for the tip area, were insulated with Epoxylite 6001 (Epoxylite, Irvine, CA).

After the electrodes were positioned into the VPMpc, the animal was mounted in the stereotaxic instrument using a hand-made, non-traumatic head holder. The animal was mounted with the snout downward 27° from horizontal to straighten the brain stem and minimize brain movement associated with breathing. A mid sagittal skin incision was made overlying the posterior skull, and a portion of the occipital bone just dorsal to the foramen magnum was removed to reveal the cerebellum. The dura covering the cerebellum was excised, and the posterior portion of the cerebellum was aspirated for 5–6 mm anterior to the obex, allowing direct access to the PbN. Body temperature was maintained at 37 ± 1°C with an electric heating pad.

Single-unit recording and electrical stimulation. Single-barrel glass micropipettes (tip diameter = 1–2 μm, resistance = 5–7 MΩ) filled with 2% (wt/vol) solution of Chicago Sky Blue dye (Sigma, St. Louis, MO) in 0.5 M sodium acetate were used for extracellular single-unit recording of action potentials from the gustatory PbN. The mean coordinates for the PbN recording were 4.0 ± 0.15 (SD) mm anterior to the obex and 1.4 ± 0.08 mm lateral to the midline. Extracellular action potentials were amplified with a band-pass of 15–5,000 Hz (NeuroLog, Digitimer, Hertfordshire, UK), discriminated with a dual time-amplitude window discriminator (Bak DD1S-1, Bak Electronics, Germantown, MD), displayed on oscilloscopes, and monitored with an audio monitor. A Dell Pentium 4 GX 280 desktop computer configured with a CED Power1401 interface board and Spike2 software (Cambridge Electronic Design, Cambridge, UK) controlled taste stimulus delivery and online data acquisition and analysis. The taste responses of the PbN neurons were initially identified by a change in neural activity associated with the application of electrical shock (≤40 μA, 500-ms duration at 1/3 Hz) to the anterior tongue, which drives gustatory fibers of the chorda tympani nerve (73) and confirmed by response to chemical stimulation of the anterior tongue. Taste stimuli presented to the anterior tongue were 0.032 M sucrose, 0.032 M sodium chloride (NaCl), 0.032 M quinine hydrochloride (QHC), and 0.0032 M citric acid. These taste solutions were delivered by a gravity-flow system composed of a computer-controlled, two-way, solenoid-operated valve connected via tubing to a distilled water rinse reservoir and a stimulus funnel. The stimulation sequence, during which the computer acquired data, was a continuous flow initiated by the delivery of distilled water for 10 s, followed by 10 s of stimulus, followed by 10 s of distilled water rinse. The flow rate was 2 ml/s. In addition to the 10-s poststimulus distilled water rinse, the tongue was rinsed with distilled water after each taste trial (>50 ml). Individual stimulations were separated by ≥2 min to avoid adaptation effects (72).

After each PbN neuron was characterized for its taste response profile, rectangular pulses (0.5 ms, ≤0.1 mA) were delivered manually to the bilateral VPMpc through each stimulating electrode from an isolated stimulator (Grass S88, Grass Instruments, Quincy, MA) to examine whether this neuron showed antidromic responses. It is known that a current of 0.1 mA activates all axons contained within a sphere of 60 μm radius and 50% of the axons located at 108 μm from the stimulating electrode (50). Given the observation that the areal dimension of the VPMpc in hamsters is ~0.77 (width) × 0.54 (height) × 0.72 (length) mm (43), a 0.1-mA current may have little influence on the structures surrounding the VPMpc. The criteria for antidromic activation were constant latency and the ability to follow a stimulus pulse pair at >100 Hz. A collision test was performed between a spontaneously generated action potential and a VPMpc stimulus-evoked potential. For the silent neurons, a collision test was performed between a taste-evoked action potential and a VPMpc stimulus-evoked potential. The latency of an antidromic response of a PbN cell was determined on storage oscilloscopes (Tektronix D13 dual-beam storage oscilloscope, Van Nys, CA, and HM 1507-3, storage oscilloscope, HAMEG Instrument, Frankfurt, Germany), as shown in Fig. 4. It was calculated from the elapsing time from the delivery of an electrical shock to the initiation of an action potential evoked by VPMpc stimulation.

To examine orthodromic responses, the VPMpc was stimulated (0.5 ms, 0.1 mA, 1/3 Hz) to observe the effect of electrical stimulation of the forebrain on ongoing activity of all PbN cells, including the VPMpc projection neurons after testing the antidromic status. A peristimulus time histogram (PSTH) was created from data acquired on each PbN cell in response to 50–200 stimulus pulses delivered to each of the two VPMpc stimulating electrodes. The latency of an orthodromic response (both inhibitory and excitatory response) was
determined by the following procedures. After a PSTH was created following the VPMpc stimulation (50–200 sweeps), the inhibitory or excitatory epoch was determined on individual PSTH (see data analysis). An individual PSTH has a time span of 1 s of 1-ms bins, and we could determine the onset of response epoch in millisecond scale by enlargement of the x-axis (time) of a PSTH using a CED software. The latency of an orthodromic response was defined as the time at which the onset of the response after the delivery of electrical shock to the VPMpc occurred (time 0).

To examine whether a VPMpc projection cell can also be driven orthodromically at a stimulus intensity that is lower than the antidromic activation threshold, the VPMpc was also stimulated with stimulus intensity at 0.8× antidromic thresholds in a subset of VPMpc projection neurons.

To test whether activation of the descending input from the VPMpc alters PbN neurons' taste responses, the responses of the PbN neurons to taste stimulation were recorded before and during the delivery of trains of constant square pulses (0.2 ms, 100 Hz) to the VPMpc. To avoid the possible cumulative effect of the train stimulation of the VPMpc, the taste trials for the recovery were performed 30 min after the VPMpc stimulation. For non-VPMpc projection neurons, the stimulus intensity was fixed at 0.1 mA. To avoid antidromic spikes mixed into taste responses, the stimulus intensities were adjusted to 0.8× antidromic thresholds for those neurons projecting to the VPMpc. The electrical stimulation started at the beginning of the delivery of taste stimuli and lasted for 10 s. We used this experimental protocol to test a subset of VPMpc cells that were excited following VPMpc stimulation.

In addition, a subset of the PbN neurons that were inhibited by VPMpc stimulation were tested for their responsiveness to the single-pulse VPMpc stimulation (0.5 ms, 0.1 mA, 1/3 Hz), while each cell was driven by taste stimulation, and a PSTH was created for each cell tested. The concentration of the taste solutions used to elicit taste responses was adjusted to actuate the PbN neurons at moderate firing activities (3.0–6.4 Hz). In the first five units tested, each effective tasteant and a mixture of all effective tastants were used separately to stimulate the tongue. Because taste responses elicited by all of these stimuli were altered by VPMpc activation, only the taste mixture was used to stimulate the tongue in the remaining test (24 additional units). The taste stimulation was repeated throughout the test period as follows: 2 s of taste solution delivery followed by a 20-s pause and then delivery of a 3-s distilled water rinse. This 25-s stimulus sequence was repeated at 10-s intervals until the end of the session to create a PSTH, as in our previous study (34). This procedure is especially useful for testing units that exhibit no or low spontaneous firing and that receive inhibitory input from the VPMpc, because this protocol lets us determine whether these neurons receive inhibitory input from the VPMpc. It is technically very difficult or impossible to observe inhibitory responses of PbN neurons to electrical stimulation of the VPMpc when a PbN cell is not firing spontaneously or is firing at very low frequencies. Second, this protocol allows us to observe whether VPMpc stimulation suppresses taste-evoked discharge of PbN taste neurons at the same time.

**Histology.** At the end of each experiment, the last recording site of the day was marked by passing a 10-μA cathodal current through the recording electrode for 10 min (5 s ON-OFF) to deposit a spot of Chicago Sky Blue dye. The stimulating sites were also marked by passing a 10-μA anodal current through the inner wire of the stimulating electrodes for 30 s. The hamster was then given a lethal overdose of urethane and perfused through the heart with 200 ml of 4% formalin containing 3% potassium ferrocyanide and ferricyanide. Brains were removed, postfixed, frozen sectioned (40 μm) in the coronal plane, and stained with Neutral Red. The recording and stimulating sites were located microscopically and plotted on standard atlas sections (43).

**Data analysis and entropy calculation.** The responses of each cell to taste stimulation of the tongue were accumulated over several stimulus sequences, to include 1) 10 s of distilled water rinse just before the stimulus; 2) 10 s of stimulus flow; and 3) 10 s of distilled water rinse just after the stimulus. The net taste response was calculated as the mean number of action potentials (imp/s) during the first 5 s of chemical stimulation minus the mean number of baseline discharge spikes during the 5-s distilled water prerinse preceding the taste delivery. Responses are reported as means ± SE. A taste response was defined as effective if it was ≥2.0 SD above the baseline discharge, which was calculated from the firing activity during the 5-s distilled water prerinse before each of four taste stimuli. For orthodromic responses of PbN cells to electrical stimulation of the VPMpc, an individual PSTH was analyzed to determine excitatory or inhibitory epochs. A baseline period was defined as the 200 ms preceding stimulation; the mean ± SD of the number of spikes per 1-ms bin during this baseline period was determined. An excitatory effect of the VPMpc stimulation was defined as an epoch of at least five consecutive bins with a mean value ≥2 SD above the baseline mean, which defines a mean response with a probability of <0.05. Inhibitory responses were defined as those with at least 40 consecutive bins with a mean <50% of baseline firing rate. Because of the slow rates of spontaneous firing of many PbN cells and their asynchronous discharge patterns, a criterion for inhibition based on variance is not practical; using 40 bins defines the inhibitory epoch as a relatively sustained decrease in firing rate (6, 27).

The entropy (H) of each neuron, which is a measure of its breadth of responsiveness, was calculated using excitatory components of responses to four standard taste stimuli by the following formula:

\[ H = -1.661 \sum_{i=1}^{4} p_i \log_2 p_i \]

where H is breadth of responsiveness, 1.661 is a scaling constant, and \( p_i \) is the proportional response to each of the four components. H ranges from 0.0 for a cell that responds exclusively to one stimulus to 1.0 for a cell responding equally to all four stimuli (75).

Each PbN cell was categorized as either a VPMpc projection neuron, if it was activated antidromically from the VPMpc stimulation, or as a nonprojection neuron otherwise. Within those two categories, cells were assigned to best-stimulus groups, according to the stimulus to which the neuron responded the most.

One-way ANOVA was used to compare differences in entropies across four best-stimulus groups. The antidromic and orthodromic activation latencies and antidromic threshold following the ipsilateral and contralateral VPMpc stimulation were compared using t-tests. In a subset of PbN taste neurons, gustatory responses before, during, and after the VPMpc stimulation were compared using repeated-measures ANOVA analysis using Statistica (StatSoft).

**RESULTS**

**Histology.** A total of 114 taste-responsive PbN neurons were recorded from 34 male hamsters. Neurons (n = 6) that did not meet the statistical criterion for taste responses or animals (n = 2) that showed the misplacement of stimulating electrodes were excluded from the analysis. Representative examples of brain sections showing recording and stimulating sites are shown in Fig. 1. Iron deposits in the bilateral VPMpc, medial to the posterior thalamic nuclear group, lateral to the subparafascicular thalamic nucleus, just ventral to the parafascicular thalamic nucleus, along with electrode penetration tracks, can be seen in coronal section of the hamster thalamus in Fig. 1A. This is the portion of the thalamus that receives afferent input from the gustatory PbN in the hamster (20) and in the rat (4, 45). A recording site marked with Chicago Sky Blue dye in the PbN is shown in Fig. 1B. The marking is located in the middle of medial PbN (MPB), medial to the superior cerebellar
peduncle (SCP), and dorsal and lateral to the mesencephalic trigeminal nucleus (Me5) at the level where the locus coeruleus (LC) is most evident on this coronal section through the hamster pons.

All recording and stimulating sites were examined and reconstructed using standard atlas sections of the hamster brain (43) (figures are not shown). The tips of the 34 stimulating electrodes on both sides were confined to the VPMpc, between the caudal end of the rhomboid thalamic nucleus and the appearance of the subparafascicular thalamic nucleus, mostly near the posterior end of the VPMpc, which corresponds to the level of the third ventricle extending to the dorsal edge of the posterior hypothalamic area. There was no difference between the electrode placement sites that evoked responses (antidromic, excitatory or inhibitory responses) in the PbN neurons and the sites that produced no response. Except for one neuron, all PbN taste-responsive neurons responded either antidromically or orthodromically after ipsilateral and/or contralateral VPMpc stimulation. All stimulating electrodes that evoked antidromic responses also produced excitatory or inhibitory responses. One stimulating site in the VPMpc that produced no effect overlapped with the sites that produced antidromic and inhibitory responses.

The location of Chicago Blue Dye markings of the last PbN neurons to be recorded in each animal (n = 34) were depicted on a standard atlas section of the hamster pons at the level of the Me5 (figure is not shown). The recording sites were concentrated in the MPB, medial to the SCP and lateral to the Me5 and the LC at the level of the Me5 and LC overlap (refer to Fig. 1B). The anterior-posterior distribution of the recording marks extended rostrally from the level at which LC is first apparent and caudally to the appearance of the accessory trigeminal nucleus. The locations of the recording sites in the PbN were similar to those of our laboratory’s previous recordings (34, 35).

The characteristics of the responses of taste neurons in the PbN. Each PbN neuron was tested for its response to the four basic taste stimuli. The responses of one cell to gustatory stimuli are shown in Fig. 2, which depicts 30 s of the neural
activity during taste trials. The overall baseline activity of the 114 taste-responsive PbN neurons recorded in the present study varied from 0 to 15.80 imp/s, with a mean of 3.58 ± 0.34 imp/s. This mean firing rate was similar to that recorded previously in the PbN (e.g., 3.70 ± 0.27 imp/s, \( t = 0.266, df = 229, P = 0.791 \)) (34) and was significantly higher than that recorded in the NST in our previous experiments (e.g., 2.12 ± 0.27 imp/s, \( t = 3.350, df = 221, P < 0.001 \)) (36).

Each gustatory neuron was categorized as NaCl, sucrose, citric acid, or QHCl best, according to its response profile. QHCl-best and sucrose-best neurons were the most and the least frequently recorded cell types, respectively (\( \chi^2 = 9.158, df = 3, P = 0.027 \)). The best-stimulus categories for each of the 114 PbN neurons are indicated in Fig. 3, where the responses of the cells in each best-stimulus group are arranged along the abscissa in order of their response to the best stimulus for that group. The response to any one tastant is read across the pattern, and that of any one neuron can be seen from top to bottom. Responses to the stimuli are net responses, and the mean baseline activity of each cell is shown as distilled H2O at the bottom of Fig. 3.

Entropy calculation showed that sucrose-best neurons were the most narrowly tuned among the four best-stimulus groups. The means of the other three groups were the same. Thus the difference of entropies among four best-stimulus groups was not significant \([F(3,110) = 2.082, P = 0.107] \). Since the projection neurons outnumbered the nonprojection cells, the comparison of taste responses or entropy between the two groups was not analyzed as it was between orthodromically responsive vs. nonresponsive cells. Mean responses to four taste stimuli and distilled water and entropy of each best-stimulus group are summarized in Table 1.

Antidromic activation of PbN cells following VPMpc stimulation. One hundred nine out of 114 taste-responsive PbN cells were activated antidromically by stimulation of ipsilateral \((n = 104, 91.2\%)\) or contralateral \((n = 77, 67.5\%)\) VPMpc. Seventy-two neurons (63.2\%) were projected bilaterally. The five nonprojection neurons were composed of two citric acid-best neurons and one each of the other three best-stimulus groups. Examples of antidromic responses of two PbN neurons induced by the ipsilateral (Fig. 4A) and contralateral VPMpc stimulation (Fig. 4B) are shown in Fig. 4, which demonstrates fulfillment of the criteria for antidromic activation.

The mean onset latency for antidromic activation following the bilateral VPMpc stimulation was 2.95 ± 0.17 ms. The mean antidromic latencies from the ipsilateral and contralateral VPMpc were 2.36 ± 0.14 ms \((range = 1.2–9.0 \text{ ms})\) and 3.74 ± 0.33 ms \((range = 1.4–16.8 \text{ ms})\), respectively. The mean onset latency for antidromic activation was significantly shorter after the ipsilateral VPMpc stimulation than after the contralateral VPMpc stimulation \((t = 4.265, df = 179, P < 0.001)\). The frequency distribution of antidromic latencies of the taste-responsive PbN cells in response to bilateral VPMpc stimulation is shown in Fig. 6A. Fifty-eight PbN neurons showed antidromic latencies between 1 and 2 ms following the ipsilateral VPMpc stimulation, whereas only six cells were in this latency range following the contralateral VPMpc stimulation. In comparison, the antidromic responses with latencies >10 ms were all induced after the contralateral VPMpc stimulation. The overall mean threshold for antidromic activation of PbN cells following the bilateral VPMpc stimulation was 51.71 ± 1.65 \(\mu A\). The mean thresholds for antidromic invasion of PbN neurons after the ipsilateral and contralateral VPMpc stimulation were 50.13 ± 2.19 \(\mu A\) \((range = 13–98 \mu A)\) and 53.84 ± 2.50 \((range = 18–100 \mu A)\), respectively. A comparison of the antidromic activation thresholds after the ipsilateral and contralateral VPMpc stimulation showed no difference \((t = 1.113, df = 179, P = 0.267)\).

Orthodromic activation of gustatory PbN neurons. Of 114 PbN neurons tested for their responsiveness to VPMpc stimulation, 110 (96.5\%) neurons, including 106 of 109 projection neurons, were orthodromically activated by stimulation of the ipsilateral or contralateral VPMpc. There were four nonresponsive neurons: one citric acid-best and three QHCl-best cells. Stimulation of ipsilateral VPMpc and contralateral VPMpc produced an orthodromic response in 101 \((88.6\%)\) and 87 \((76.3\%)\) neurons, respectively; 78 \((68.4\%)\) cells responded orthodromically to both sides of the VPMpc. Among orthodromic activation, inhibition was the predominant response. The neuronal activity of 106 cells was inhibited by the VPMpc stimulation; 68 showed bilateral inhibition. The excitatory responses were observed in 14 PbN cells; 4 neurons responded unilaterally and 10 neurons also showed inhibitory responses to the stimulation of the other side of the VPMpc. Among these 10 cells, 7 cells were inhibited during the ipsilateral VPMpc
stimulation, but excited during stimulation of the contralateral VPMpc, and 3 cells were excited during the ipsilateral VPMpc stimulation, but inhibited during stimulation of the contralateral VPMpc. All of these PbN cells were VPMpc projection cells (see Table 2). A few NST taste neurons showed a similar response pattern following bilateral BNST or LH/CeA stimulation, in our laboratory’s previous studies (6, 76). Examples of orthodromic responses induced by ipsilateral and contralateral stimulation are shown in Fig. 5. We were able to measure the orthodromic response latencies and durations of inhibition or excitation of 51 PbN neurons: 34 after the ipsilateral VPMpc stimulation and 39 after the contralateral VPMpc stimulation. The mean response latency of inhibition and excitation were 21.52 ± 1.55 ms (range = 9–65 ms) and 35.69 ± 6.89 ms (range = 15–80 ms), respectively. The mean duration of inhibition and excitation were 89.72 ± 5.76 ms (n = 60, range 47–219 ms) and 33.23 ± 8.69 ms (n = 13, range 5–110 ms), respectively. The frequency distribution of orthodromic response latencies of the taste-responsive PbN cells in response to bilateral VPMpc stimulation is shown in Fig. 6B. The difference in the inhibitory latencies was not significant between the ipsilateral (mean = 20.13 ± 1.98 ms, n = 30) and contralateral VPMpc stimulation (mean = 22.90 ± 2.39 ms, n = 30) (t = 1.281 df = 29, P = 0.211).

Stimulation of the VPMpc orthodromically activates VPMpc-projecting PbN neurons. To examine whether VPMpc stimulation orthodromically activates PbN gustatory neurons that project to the VPMpc, we raised the stimulus intensity up to 100 μA and stimulated the VPMpc again at 1/3 Hz after examining the antidromic response status. One hundred one of 104 (97.1%) ipsilateral VPMpc-projecting neurons and 74 of 77 (96.1%) contralateral VPMpc-projecting neurons showed orthodromic responses at 100 μA. Examples of VPMpc projection neurons that were orthodromically activated after VPMpc stimulation are shown in Fig. 7, A and C. To determine whether VPMpc projection cells can also be driven orthodromically at stimulus intensities lower than the antidromic activation thresholds, a subset of PbN projection neurons was stimulated at subthreshold stimulus intensities that were required for antidromic activation. A PSTH was created for each neuron tested in response to 50–200 stimulus pulses. We found that 41.5% of VPMpc projection neurons showed orthodromic responses, even at the subthreshold intensities. Twelve neurons out of 30 were orthodromically activated following the ipsilateral VPMpc stimulation at subthreshold intensity, as were 22 of 52 neurons activated after the contralateral VPMpc stimulation. Examples of two VPMpc-projecting PbN neurons that were activated following the ipsilateral and contralateral VPMpc stimulation are shown in Fig. 7. The distribution of the PbN neurons as a function of the antidromic and orthodromic responsiveness is demonstrated in Table 2. The number of PbN cells in orthodromic response categories is based on response at 100 μA.

Effects of electrical stimulation of the VPMpc on taste response of PbN neurons. The majority of orthodromic responses of gustatory PbN neurons to VPMpc stimulation were
inhibition. It was difficult to observe inhibitory responses in some PbN neurons because of their low spontaneous firing rate. In such cases, the inhibitory effect could be detected by measuring the change of taste stimulation-evoked neuronal activity to electrical stimulation of the VPMpc (100 μA, 1/3 Hz). This procedure also demonstrates the inhibitory modulation of taste response of the tested neuron by VPMpc simultaneously. Twenty-nine PbN neurons (15 cells to ipsilateral and 14 to contralateral VPMpc stimulation), composed of 5 sucrose-best, 7 NaCl-best, 6 citric acid-best, and 11 QHCl-best neurons that receive inhibitory input from the VPMpc, were examined, and a PSTH was created for each cell tested. Examples of the influence of the VPMpc stimulation on taste responses are shown in Fig. 8. VPMpc activation suppressed both spontaneous and taste-evoked spikes, as shown in PSTHs in all cells tested.

To test the influence of activation of VPMpc on the taste responses of PbN neurons that received excitatory input from the VPMpc, taste responses of PbN neurons before and during electrical stimulation of the VPMpc were compared. Taste responses of 8 out of 13 PbN neurons that were excited by the VPMpc stimulation were successfully examined with this protocol. An example of VPMpc stimulation effect on taste responses of a PbN neuron is demonstrated in Fig. 9A. This neuron responded best to citric acid and not to sucrose and was excited by VPMpc stimulation. Gustatory response to NaCl, citric acid, and QHCl was enhanced during the VPMpc stimulation, but response to sucrose was still absent. Taste responses to citric acid, NaCl, and QHCl increased by 77, 71, and 59%, respectively, during VPMpc activation. The overall gustatory responses of eight PbN cells are demonstrated in Fig. 9B. Taste responses were significantly increased when gustatory stimuli were delivered during the VPMpc stimulation $[F(2,56) = 30.96, P < 0.0001]$. The mean taste responses without or with VPMpc stimulation were 7.31 ± 1.27 and 10.59 ± 1.74 imp/s, respectively. Taste responses had returned to the control level when tested 30 min after the VPMpc stimulation (mean response = 7.58 ± 1.34 imp/s). The enhancement of the gustatory responses during VPMpc stimulation was nonspecific; the effect of the stimulus was not significant $[F(3,56) = 1.73, P = 0.184]$. The interaction between the stimulus and VPMpc stimulation was not significant either $[F(6,56) = 1.47, P = 0.205]$. The planned comparison showed that responses to NaCl $[F(1,28) = 12.613, P < 0.005]$, to citric acid $[F(1,28) = 8.620, P < 0.01]$, and to QHCl $[F(1,28) = 19.661, P < 0.001]$ significantly increased during the VPMpc stimulation.

DISCUSSION

The main finding of the present investigation was that stimulation of the bilateral VPMpc inhibited almost all of gustatory PbN neurons; only a small number of cells were excited (12.3%) or did not respond to the VPMpc stimulation (3.5%). The possible role of the VPMpc activation was to change the magnitude of response of PbN neurons to taste stimuli. We also demonstrated that the majority of gustatory PbN neurons project to the VPMpc bilaterally in hamsters.

Afferent projection of PbN gustatory neurons to the VPMpc. In the present study, we recorded from 114 taste-responsive PbN neurons and tested their responsiveness to the VPMpc.
stimulation. The distributions of recording markings were within the MPB and concentrated between SCP and LC/Me5 at the level where the LC and Me5 overlap. The locations of these recording sites correspond to the area where PbN receives its predominant input from the gustatory NST in the hamster (88), and the distribution characteristic is similar to that shown when our laboratory recorded taste-responsive PbN neurons in previous investigations (34, 35, 80, 82). The mean baseline discharge rate was also similar to the firing rates our laboratory recorded from PbN taste neurons in our previous studies (34, 35).

Neuroanatomical studies have shown that neurons in the gustatory PbN project to the VPMpc in both rats and hamsters. An early tract tracing study in the rat, using tritiated proline autoradiography, has shown that labeled axons of third-order gustatory neurons in the dorsal pons project to the VPMpc and their terminal fields extend anteromedially across the midline into the contralateral VPMpc (47). In the hamster, after microinjection of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) into the MPB, the labeled fibers traveled rostrally from the injection site via the ipsilateral central tegmental tract, and the major termination site of the labeled fibers were within both sides of the VPMpc (21). The projections of taste neurons in the PbN to the VPMpc have also been demonstrated electrophysiologically. Norgren (47) demonstrated that a subset of NaCl and/or thermal-responsive PbN neurons in the rat were antidromically driven by stimulation of the ipsilateral amygdala and/or VPMpc. Ogawa and colleagues (51, 52) reported in two separate studies that 55–59% of taste neurons in the PbN project to either or both of the ipsilateral or contralateral VPMpc in the rat.

In the present study, we demonstrated that 91.2 and 67.5% of gustatory PbN neurons project to the ipsilateral and con-

Fig. 7. PSTHs depicting the responses of two VPMpc projection PbN neurons following the ipsilateral (A and B) and contralateral VPMpc (C and D) stimulation. Note that the inhibitory responses of these two neurons were induced by VPMpc stimulation at suprathreshold stimulus intensities (A and C; antidromic responses were followed by inhibitions) and subthreshold stimulus intensities (B and D; inhibitory responses without evoking antidromic spikes) for antidromic activation. Electrical pulses were delivered to the VPMpc at time = 0. PSTHs were accumulated over 200 stimulus sweeps at 1/3 Hz.

Fig. 8. PSTHs illustrating the effect of stimulation of the ipsilateral (A and B) and contralateral VPMpc (C and D) stimulation on firing activity of two PbN neurons created without (A and C) or with (B and D) chemical stimulation of the tongue. Note that, with the same number of stimulus impulses, a much greater number of spikes were accumulated, and the inhibitory effect is evident in the PSTHs produced with chemical stimulation of the tongue. Electrical stimulation was delivered to the VPMpc at time = 0. PSTHs were accumulated over 100 (A and B) or 200 (C and D) stimulus sweeps at 1/3 Hz. Chemical stimulation for the PSTHs (B and D) was a mixture of effective stimuli.
tralateral VPMpc, respectively. These results confirmed earlier tract tracing studies and the reports of Ogawa and colleagues (51, 52) that taste neurons in the PbN project to the VPMpc bilaterally. We found that a much higher proportion of taste-responsive PbN neurons project to either or both of the ipsilateral or contralateral VPMpc (95.6%) than were reported by Ogawa and colleagues (55–59%). This difference may result from species differences. An interspecies difference between rat and hamster has also been reported in the proportion of afferent projection of second-order gustatory relay neurons to the PbN. For instance, it was reported that 34.1% (53) or 45% (42) of the taste neurons in the NST project to the PbN in the rat, whereas a larger percentage (80.2%) was reported in an investigation with hamsters (7).

Whether antidromic responses of PbN taste neurons following the VPMpc stimulation were due to stimulation of the axonal terminals or the fibers of passage within the VPMpc is beyond the scope of the present study. Antidromic stimulation can only specify where axons originate, not where they terminate. It has been reported that, following microinjection of [3H]proline into the gustatory PbN, labeled axonal terminals and some passing fibers were observed in the VPMpc in the rat (47). Therefore, antidromic responses of the PbN cells after the VPMpc stimulation may result from activation of both cell terminals and passing fibers.

Anatomical studies have shown that cells in the gustatory PbN project heavily to the VPMpc and ventral forebrain gustatory areas, both in the hamster (21) and in the rat (48). It was reported that many PbN taste cells project both to the VPMpc and ventral forebrain; 10 of 21 PbN taste cells that were antidromically activated from the VPMpc were also antidromically invaded from the electrodes aimed in the ventral forebrain (45), suggesting that some PbN cells are bifurcating. The existence of collaterals of axons of PbN taste cells may partially explain the high antidromic activation from the VPMpc.

The mean antidromic activation latencies of PbN cells following the ipsilateral (2.36 ± 0.14 ms) and contralateral VPMpc stimulation (3.74 ± 0.33 ms) were similar to those in the rat (e.g., 2.8 ± 1.9 ms from the ipsilateral VPMpc and 3.3 ± 1.9 ms from the contralateral VPMpc) (52), with one difference. We found that the mean antidromic latency of the PbN cells following the ipsilateral VPMpc stimulation was significantly shorter than that following the contralateral VPMpc stimulation, whereas Ogawa and colleagues (52) did not show any difference, although they found that there is a tendency for neurons projecting bilaterally to have shorter antidromic latencies than neurons projecting unilaterally.

Efferent projection from the VPMpc to the gustatory PbN neurons. Neuroanatomical studies suggest that there is no direct efferent neural pathway connecting VPMpc and PbN. The possibility of monosynaptic projection from the thalamus to the pontine taste area has been ruled out, because lesions in the thalamic taste area resulted in no degenerating fibers reaching the pons (48). Halsell (21) also reported that, following microinjection of WGA-HRP into VPMpc, retrogradely labeled cell bodies were located in both the ipsilateral and contralateral PbN, with fewer cells labeled contraterally, but no anterogradely transported label was visible in the PbN. The GC, which is the main target of the VPMpc (29, 94), also projects to the PbN (29, 57, 63, 83, 88, 91, 94).

In the present study, 110 of 114 PbN cells (96.5%), including 106 of 109 VPMpc projection PbN cells, were activated orthodromically following either unilateral or bilateral VPMpc stimulation, suggesting that gustatory PbN neurons are under massive control from VPMpc. Furthermore, inhibitory response was the dominant response among orthodromic activation. These results are quite different from, but do not contradict, the neuroanatomical observations. Our electrophysiological results suggest that the efferent projection from the VPMpc to the taste neurons in the PbN is not monosynaptic. For example, the mean activation latency of the orthodromic responses of the PbN cells after the VPMpc stimulation was much longer (35.69 ± 6.89 ms for excitation and 21.52 ± 1.55 ms for inhibition) than the mean latency for antidromic activation of the PbN cells induced by VPMpc stimulation (2.95 ± 0.17 ms), suggesting that the descending projection is indirect. This analysis is supported by both tract tracing and electrophysiological studies. It was reported that, following microinjection of WGA-HRP into the VPMpc, anterogradely labeled fibers were distributed in the amygdala and BNST (21). We showed in a previous study that the vast majority of PbN taste cells receive descending input from the BNST, and every PbN cell responded orthodromically to the BNST stimulation with inhibition (34). Similar to the BNST study, VPMpc stimulation also induces an inhibitory response in the majority of PbN.
neurons. When the CeA was electrically stimulated, excitatory and inhibitory response was comparable, 12 vs. 14 out of 83 PbN cells in the hamster, and this descending input altered magnitude of gustatory responses (35). The percentage of the orthodromically activated PbN taste neurons might be higher than the reported number, because the orthodromic responsiveness was only investigated in non-CeA projection neurons in that study (35). Although stimulation parameters were different from those in the hamster, the CeA stimulation inhibited gustatory response in 34 PbN cells out of 51 in the rat (40). Whereas Lundy and Norgren (39) reported electrical stimulation of the GC more often inhibited gustatory responses of the PbN neurons, Di Lorenzo (10) observed excitation in 17 out of 19 PbN taste neurons, which responded to GC stimulation in the rat.

In addition, neuroanatomical studies demonstrated that axons from neurons in the CeA, BNST, or GC terminate within the gustatory PbN, but not from the VPMpc (83, 88).

Taken together, these data may suggest that the descending pathway from the VPMpc to the taste cells in the PbN may project first to the CeA, BNST, and/or GC. Further studies involving local anesthetization and chemical stimulation (e.g., microinjection of Nα-homocysteic acid) of the CeA, BNST, and/or GC is necessary to prove this descending pathway.

As discussed for the antidromic responses of the PbN neurons induced following the VPMpc stimulation, we cannot completely rule out the possibility that orthodromic responses of PbN taste neurons following the VPMpc stimulation were due to direct stimulation of fibers destined for ventral forebrain structures, since the fibers projecting to the ventral forebrain pass very close to the ventral border of the VPMpc (44, 63). Although the possibility of current spreading to the adjacent structures was minimal, since the current applied to the VPMpc was small (≤100 μA), the electrode tips that were positioned near the ventral border of the VPMpc may have activated the fibers passing very close to the ventral border of the VPMpc. A combination of electrical stimulation and chemical stimulation is necessary to clarify this question.

VPMpc stimulation facilitates VPMpc projection PbN taste neurons. We demonstrated previously that the majority of BNST projection PbN neurons receive descending input from the BNST; all 13 BNST projection PbN cells were inhibited (34). In the present study, we tested whether VPMpc projection PbN cells also can be orthodromically activated from the VPMpc. We confirmed that VPMpc stimulation orthodromically activates 106 of 109 (97.2%) VPMpc projection PbN neurons, which were largely inhibitory, except for 13 cells from the VPMpc.

Because the orthodromic activation of VPMpc projection neurons was induced by suprathreshold stimulus intensity following antidromic spikes (Fig. 7, A and C), we could not rule out the possibility that the orthodromic responses were due to an absolute refractory period of the antidromic spike or recurrent inhibition. To examine the relationship between the antidromic responses and the orthodromic responses, a subset of projection neurons that showed orthodromic responses was further tested with the stimulus intensities lower than the threshold for antidromic activation. Forty-one and one-half percent of the units tested were orthodromically activated with this protocol. These neurons had relatively higher thresholds for antidromic activation. Because the orthodromic responses of these neurons were induced without eliciting antidromic spikes, it is likely that antidromic action potentials were not involved in this orthodromic activation of these PbN cells; rather, it suggests that there is a separated reciprocal circuit between taste neurons in the PbN and VPMpc. The relationships between antidromic and orthodromic responses for the VPMpc projection PbN cells that were not activated orthodromically with subthreshold stimulus intensities required for antidromic activation remains unknown. However, given the fact that the antidromic activation thresholds for most of these cells were relatively low, cells that were not responding orthodromically with this testing scheme may, due to the stimulus intensities, be below the orthodromic activation thresholds.

Anatomical study has shown that, following the microinjection of WGA-HRP into the PbN, labeled fibers travel rostrally via the ipsilateral central tegmental tract, and the major termination sites of these fibers were within both the ipsilateral and contralateral VPMpc (21). In the same study, the anterogradely labeled fibers were observed in the ipsilateral CeA and the BNST, but not in the PbN, following the injection of neural tracer into the VPMpc in hamsters (21). The projection from the VPMpc to the CeA has also been reported in the rat (81). The injection of WGA-HRP or Phaseolus vulgaris leucoagglutinin into the BNST and CeA labeled cells and fibers in the PbN (41). Taken together, the efferent projection from the VPMpc may project to the amygdala and the BNST before reaching the gustatory PbN, whereas some PbN neurons send their axons to the bilateral VPMpc directly. These results demonstrate that VPMpc projection PbN taste neurons not only send their axons to the VPMpc, but also receive indirect descending inputs from the VPMpc. This further signifies the importance of the VPMpc as the relay of the thalamocortical taste pathway.

Activation of VPMpc alters taste responses of the neurons in the PbN. As presented above, a large majority of the taste-responsive PbN cells were under the control of efferent projection from the VPMpc. To test further whether stimulation of VPMpc alters taste responses of the PbN neurons, we examined the influence of electrical stimulation of the VPMpc on taste responses of PbN neurons. In the first experiment, we tested whether a single-pulse stimulation of VPMpc inhibits taste stimulus-elicited neuronal activity in a subset of PbN neurons that receive inhibitory input from the VPMpc. As shown in PSTHs in Fig. 8, B and D, electrical stimulation of the VPMpc suppressed activity of the PbN cells. Since the spikes appearing in the PSTHs (Fig. 8, B and D) were mostly driven by taste stimulation, VPMpc-induced inhibition represents the suppression of both spontaneous and taste stimulation-elicited responses. In the second set of experiments, taste responses were compared before and during trains of electrical stimulation of the VPMpc in the PbN neurons that receive excitatory input from the VPMpc. In this protocol, each of four taste stimuli was applied to the anterior tongue three times: before (or control), during, and 30 min after the electrical stimulation of the VPMpc. Only the magnitude of responses to effective taste stimuli was increased with the VPMpc stimulation. This enhancement was observed regardless of stimulus, as long as it is effective to produce response per se, and the best-stimulus category was maintained during the VPMpc stimulation. Taken together, these experiments demonstrate...
that VPMpc activation modulates taste responses of PbN neurons that were excited or inhibited by VPMpc stimulation.

Modulation of taste responses of NST or PbN neurons by activation of descending pathways has been previously reported. Electrical stimulation of the BNST modulated taste responses in the NST and PbN (34, 74). Activation of the CeA and LH also modulated taste responses of the neurons in the NST and PbN (8, 35, 36). Modulation of PbN neurons’ taste responses by forebrain activation, including the CeA, LH, and GC, has also been demonstrated by Lundy and Norgren (39, 40). CeA or GC stimulation generally changed gustatory responses of PbN neurons nonspecifically in the control and CTA groups, or GC stimulation generally changed gustatory responses of PbN neurons nonspecifically in the control and CTA groups, except for amiloride-sensitive, NaCl-best neurons following the CeA stimulation in the rat (79).

**Perspectives and Significance**

The VPMpc is positioned at the key location between the gustatory PbN and insular cortex for relaying and processing gustatory information via the thalamocortical pathway. The present study further confirmed the strong afferent connection from the tongue to the thalamus, which is consistent with previous studies (8, 34–36, 74). The effect of activation of efferent taste pathway on the responses of gustatory PbN neurons was also nonspecific in the rat, but the net effect sharpened the response to best stimulus of those neurons (39, 40). CeA or GC stimulation generally changed gustatory responses of PbN neurons nonspecifically in the control and CTA groups, except for amiloride-sensitive, NaCl-best neurons following the CeA stimulation in the rat (79).

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