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Descending projections from the nucleus accumbens shell suppress activity of taste-responsive neurons in the hamster parabrachial nuclei

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Li C-S, Chung S, Lu D, Cho YK. Descending projections from the nucleus accumbens shell suppress activity of taste-responsive neurons in the hamster parabrachial nuclei. *J Neurophysiol* 108: 1288–1298, 2012. First published June 13, 2012; doi:10.1152/jn.00121.2012.—The parabrachial nuclei (PbN), the second central relay for the gustatory pathway, transfers taste information to various forebrain gustatory nuclei and to the gustatory cortex. The nucleus accumbens is one of the critical neural substrates of the reward system, and the nucleus accumbens shell region (NAcSh) is associated with feeding behavior. Taste-evoked neuronal responses of PbN neurons are modulated by descending projections from the gustatory nuclei in the forebrain. In the present study, we investigated whether taste-responsive neurons in the PbN project to the NAcSh and whether pontine gustatory neurons are subject to modulatory influence from the NAcSh in urethane-anesthetized hamsters. Extracellular single-unit activity was recorded in the PbN, and taste responses were confirmed by the delivery of 32 mM sucrose, NaCl, quinine hydrochloride, and 3.2 mM citric acid to the anterior tongue. The NAcSh was then stimulated (0.5 ms, $\leq$100 μA) bilaterally using concentric bipolar stimulating electrodes. A total of 98 taste neurons were recorded from the PbN. Eighteen neurons were antidromically invaded from the NAcSh, mostly the ipsilateral NAcSh (n = 16). Stimulation of the ipsilateral and contralateral NAcSh suppressed the neuronal activity of 88 and 55 neurons, respectively; 52 cells were affected bilaterally. In a subset of pontine neurons tested, electrical stimulation of the NAcSh during taste stimulation also suppressed taste-evoked neuronal firing. These results demonstrated that taste-responsive neurons in the PbN not only project to the NAcSh but also are under substantial descending inhibitory influence from the bilateral NAcSh.

reward; gustatory; in vivo electrophysiology

PALATABLE FOODS, such as sucrose, fat, or alcohol, are often eagerly consumed, and overconsumption may result in obesity or alcohol abuse. Exceeding intake of palatable food more than the homeostatic need is governed not only by the taste system but also by the reward system (Berridge 1996; Norgren et al. 2006). The nucleus accumbens (NAcc), which is located in the ventral forebrain, is a neural structure mediating reinforcement and motivational behavior (Berridge and Robinson 1998; Norgren et al. 2006); the NAcc shell region (NAcSh) especially plays an important role in regulating behavior in response to palatable stimuli (Di Chiara 2002; Loriaux et al. 2011; Norgren et al. 2006). Anatomically, the NAcc is closely connected with various brain sites. The ventral tegmental area, lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST) send axons to the NAcc, and axons from the NAcc terminate in the ventral tegmental area, ventral pallidum, and LH (Berridge et al. 1997; Kelley et al. 2005; Sano and Yokoi 2007; Swanson 2000; Wise and Rompre 1989; Wood and Swann 2005). Recent studies have suggested a role of the NAcc in regulating ingestive behavior. Sucrose intake induced dopamine release in the NAcc (Doyon et al. 2003; Hajnal et al. 2004; Liang et al. 2006; Norgren et al. 2006; Weiss et al. 1993). Dopamine release in the NAcc was also observed after cocaine or alcohol ingestion (Carboni et al. 2001; Weiss et al. 1993). Injection of glutamate antagonists or GABA agonists into the NAcSh elicited feeding in satiated rats (MacDonald et al. 2003, 2004; Maldonado-Irizarry and Kelley 1994, 1995; Stratford and Kelley 1997). Opioid receptor agonist-evoked food intake was reduced by pretreatment of the NAcSh with opioid receptor antagonist (Ragnauth et al. 2000). These studies have also suggested that the involvement of the NAcSh in controlling feeding behavior is possibly driven by the hedonic value of food but not homeostatic needs.

Physiological factors associated with nutritional homeostasis, such as blood insulin and glucose levels, gastric distention, sodium appetite, and taste aversion, can alter taste-evoked neuronal activities of some neurons in the nucleus of the solitary tract (NST) (Chang and Scott 1984; Giza et al. 1993, 1997; Giza and Scott 1983, 1987; Gleen and Erickson 1976) or in the parabrachial nuclei (PbN) (Baird et al. 2001; Cho et al. 2004; Shimura et al. 1997; Tamura and Norgren 1997). Most forebrain gustatory nuclei, such as in the LH, CeA, and ventral posteromedial nucleus (VPM), are involved in regulating food-related behaviors (Berridge and Valenstein 1991; Delgado and Anand 1953; Flynn et al. 1991; Ganaraj and Jeganathan 1998; Grigson et al. 1997; Grossman and Grossman 1982; Norgren 1970; Reilly 1998; Seeley et al. 1993). Taste-responsive neurons in the NST and PbN are under the influence of those rostral gustatory nuclei; electrical stimulation of the LH, CeA, BNST, VPM, and gustatory cortex excites and/or inhibits neuronal firing to taste stimulation in some neurons in the rat and hamster (Cho and Li 2008; Cho et al. 2002a; 2002b; 2003, 2008; Li and Cho 2006; Li et al. 2002, 2005, 2008; Lundy and Norgren 2001, 2004a; Mao et al. 2008). However, whether the

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NAcSh may exert an influence on gustatory information processing in the taste system has not been investigated. The purpose of the present study was to examine the direct functional relationship between the reward system (NAcSh) and gustatory system (PbN) using in vivo electrophysiological techniques.

METHODS

Animal care and surgery. Experimental procedures were conducted in accordance with the guidelines on the use and care of laboratory animals set by the Institutional Animal Care and Use Committee of Southern Illinois University and the Animal Use Committee of Gangneung-Wonju National University, which approved the protocols used in this study. Young adult male Syrian golden hamsters (*Mesocricetusauratus*, $n = 33$), weighing between 121 and 170 g, were deeply anesthetized with urethane (1.7 g/kg ip). Additional anesthetic (10% of the original dose) was given as needed during the course of each experiment to maintain anesthesia. Each animal was tracheotomized, and the bilateral hypoglossal nerves were cut at the neck to prevent tongue movement. The animal was then 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 frontal bone was removed, and a hole was drilled on each side of the skull to access the NAcc bilaterally. A concentric bipolar stimulating electrode, constructed from 26-gauge stainless steel tubing and 140-μm-thick stainless steel wire, was lowered into the NAcSh on each side of the brain (2.65 mm anterior to the bregma, 1.1 mm lateral to the midline, and 5.4 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 NAcSh, the head of the animal was bent downward 27° from the horizontal position using a handmade nontraumatic head holder to straighten the brain stem. This position minimizes brain movement associated with breathing and the heart beating. A sagittal skin incision was made along the midline overlying the posterior skull, and the underlying soft tissue was excised. A portion of the occipital bone between the posterior edge of the foramen magnum and just posterior to the interparietal bone was removed to reveal the cerebellum. The dura covering the cerebellum was excised, and the posterior portion of the cerebellum was aspirated bilaterally 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 (Watlow, St. Louis, MO).

Single-unit recordings in the PbN and taste stimulation. Single-barrel glass micropipettes (tip diameter: 1–2 μm, resistance: 5–7 MΩ) filled with a 2% (wt/vol) solution of Chicago blue dye (Sigma) in 0.5 M sodium acetate were used for extracellular recordings of single-unit action potentials from the gustatory PbN. The coordinates (means ± SD) for the PbN recordings were 4.0 ± 0.16 mm anterior to the obex and 1.4 ± 0.09 mm lateral to the midline. Extracellular action potentials were amplified with a band pass of 15–5,000 Hz (NeuroLog, Digitimer, Hertfordshire, UK), isolated with a dual time-amplitude window discriminator (Bak DDIS-1, Bak Electronics, Germantown, MD), displayed on oscilloscopes, and monitored with an audio monitor. A computer configured with a CED 1401 interface board and Spike2 software (Cambridge Electronic Design, Cambridge, UK) was used for taste stimulus delivery, data acquisition, and online data analysis.

Gustatory PbN neurons were initially identified by a change in neural activity associated with the application of electrical shock ($\leq$40 μA, 500-ms duration at 1/3 Hz) to the anterior tongue and then confirmed by responses to chemical stimulation of the anterior tongue. Taste stimuli presented to the anterior tongue were as follows: 0.032 M sucrose, NaCl, quinine hydrochloride (QHCl), and 0.0032 M citric acid. These concentrations evoke approximately equal multiunit taste responses in the hamster NST (Duncan and Smith 1992). The taste solution was delivered by a gravity-flow system composed of a computer controlled two-way solenoid-operated valve connected to a distilled water rinse reservoir and a stimulus funnel. The stimulation sequence, synchronized with data acquisition, was a continuous flow initiated by the delivery of distilled water for 10 s followed by 10 s of a taste solution and lastly by 10 s of a distilled water rinse. The flow rate was 2 ml/s. After each tastant, the tongue was rinsed with distilled water ($>$50 ml). Individual stimulations were separated by $\geq$2 min to avoid potential interactions, including adaptation. Each cell was categorized as responding best to sucrose, NaCl, citric acid, or QHCl on the basis of its taste profile (Frank 1973).

NAcSh electrical stimulation and gustatory PbN cell characterization. After its taste response profile was characterized, each PbN neuron was then classified as a NAcc-projecting or nonprojecting cell by its antidromic responses status to electrical stimulation of the NAcSh. Rectangular pulses (0.5 ms, $\leq$0.1 mA) were delivered to the NAcSh through stimulating electrodes using an isolated stimulator (Grass S88, Grass Instruments, Quincy, MA). The criteria for antidromic activation were a constant latency and the ability to follow a stimulus pulse pair at $>$200 Hz. Finally, a collision test was performed between a spontaneously generated action potential and a NAcSh stimulus-evoked potential (Iggo 1958). The ipsilateral NAcSh was stimulated first and then contralateral NAcSh stimulation followed or vice versa. Only those PbN neurons that satisfied all the criteria for antidromic activation including the collision test were classified as NAcc-projecting cells.

After determining the antidromic response status of each neuron, we tested whether these taste-responsive PbN cells, including NAcc-projecting neurons, receive descending input from the ipsilateral or contralateral NAcSh. For this, the ipsilateral and contralateral NAcSh were stimulated in random order (0.1 mA, 0.5 ms at 1/3 Hz), and the effect of electrical stimulation on the ongoing activity of PbN neurons was observed. A peristimulus time histogram (PSTH) was created from the data acquired from each PbN cell in response to 50–200 stimulus pulses delivered to the NAcSh.

The effect of activation of the descending input from the NAcSh on taste responses was also checked in a subset of PbN neurons; taste responses were compared before and during the delivery of trains of constant square pulses (0.1 mA, 0.2 ms at 100 Hz) to the NAcSh in a total of 16 PbN taste neurons. The electrical stimulation was delivered during 10 s of taste stimulation. To observe the recovery, taste trials

Table 1. *Classification of gustatory neurons as a function of best stimulus and NAcSh stimulation-evoked response*

	Sucrose Best	NaCl Best	Citric Acid Best	Quinine Hydrochloride Best	Total
Number of neurons	25	20	30	23	98
Best stimulus					
Antidromic response	9 (1*)	3 (1*)	3	1	16 (2*)
Bilateral inhibition	14	13	14	11	52
Unilateral inhibition	10	6	15	5 (3†)	36 (3†)
No inhibitory response	1	1	1	4	7

Numbers of cells that were antidromically activated (*) or inhibited (†) by stimulation of the contralateral shell region of the nucleus accumbens (NAcSh) are shown in parentheses.

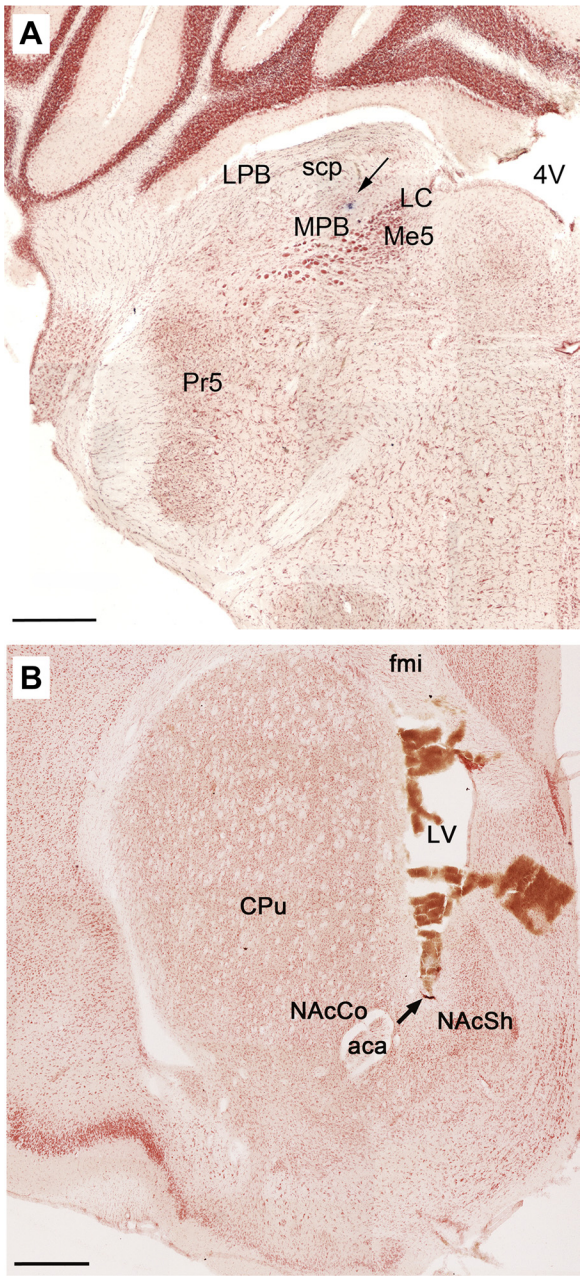

Fig. 1. Photomicrographs of coronal brain sections showing the location of recording and stimulating sites. *A*: coronal section through the pons showing a recording site (arrow) in the parabrachial nuclei (PbN) marked with Chicago blue dye. *B*: coronal section through the ventral forebrain showing the location of the ipsilateral stimulation site (arrow). An iron deposit from the tip of the stimulating electrode along with tissue damage indicate placement in the shell region of the nucleus accumbens (NAcSh). Pr5, principal sensory trigeminal nucleus; LPB, lateral parabrachial nucleus; scp, superior cerebellar peduncle; MPB, medial parabrachial nucleus; LC, locus coeruleus; Me5, mesencephalic trigeminal nucleus; 4V, fourth ventricle; CPu, caudate putamen; NAcCo, core of the nucleus accumbens; aca, anterior commissure (anterior part); fmi, forceps minor of the corpus callosum; LV, lateral ventricle. Scale bars = 500 μm in both *A* and *B*.

were repeated without NAcSh activation 15–20 min after the NAcSh activation trials. Since responses of taste-responsive PbN cells to activation of descending input from the NAcSh were exclusively inhibitory (see RESULTS), it is very difficult to observe the change in neuronal firing in response to the NAcSh stimulation if a PbN cell is not firing spontaneously or firing at a very low frequency. In such PbN neurons (n = 17), we tested for their responsiveness to the NAcSh stimulation (0.1 mA, 0.5 ms at 1/3 Hz) while elevating the firing of PbN cells by taste stimulation. The concentrations of the taste solutions were adjusted so as to elevate the PbN neuron firing at a moderate rate of 3.5~7.2 Hz. The taste stimulation that consisted of effective taste solution(s) for each neuron 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 that could be used to determine the effect of NAcSh stimulation. This experimental protocol was used to determine whether these neurons receive inhibitory input from the NAcSh and to observe whether NAcSh stimulation suppresses taste-evoked discharge of PbN neurons simultaneously. Using the same protocol, we successfully observed the inhibitory effect of low-discharging gustatory PbN neurons to BNST stimulation (Li and Cho 2006).

Histology. At the end of each experiment, the last recording site 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. Stimulating sites on the NAcSh were also marked by passing 10-μA anodal current through the inner wire of the stimulating electrodes for 20–30 s to deposit a spot of iron. The hamster was then given a lethal dose of sodium pentobarbital and perfused through the heart with 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.

Data analysis. Data collected at Southern Illinois University were used for analysis in the present study. Histological examination of stimulating electrode placement in the NAcSh revealed proper electrode placement in 29 animals. The data collected from these animals were used for analysis. Animals with misplacement of the NAcSh stimulating electrode (n = 4) were excluded from the study. Responses of each cell to taste stimulation of the tongue were accumulated from 30-s periods that consisted of 10 s of a distilled water rinse, 10 s of taste stimulus, and 10 s of a distilled water rinse. The net taste response was calculated as the mean number of action potentials (in impulses/s) during the first 5 s of taste stimulation minus the mean number of spikes during the 5 s of distilled water before the taste delivery. Responses are reported as means ± SE. A taste response was considered significant if it was ≥2 SD above the baseline discharge, which was calculated from the firing activity during the 5-s distilled

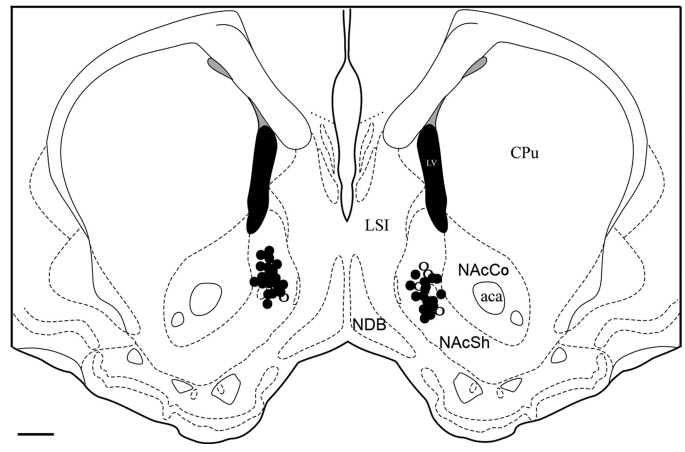

Fig. 2. Standard atlas section of the hamster through the ventral forebrain showing the distribution of the tips of stimulating electrodes in the bilateral NAcSh for the 29 experimental animals. Filled circles indicate the stimulating sites that induced antidromic and/or orthodromic responses, whereas open circles indicate the stimulating sites that produced no response. LSI, lateral septal nucleus (intermediate part); NDB, nucleus of the diagonal band of Broca. Scale bar = 500 μm.

water prerinse before each of four taste stimuli. For orthodromic responses of PbN cells to electrical stimulation of the NAcSh, an individual PSTH was analyzed to determine the excitatory or inhibitory epoch. A baseline period was defined as the 200 ms preceding the stimulation. The mean ± SD of the number of spikes/1-ms bin during the baseline period was determined. An excitatory effect of NAcSh stimulation was defined as an epoch of at least five consecutive 1-ms bins with a mean >2 SD of the baseline firing rate. An inhibitory effect was defined as at least 40 consecutive 1-ms bins in which the mean was <50% of the baseline firing rate. This definition showed a relatively sustained decrease in firing rate in a previous study (Mao et al. 2008).

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:

$$H = -1.661\sum_{i=1}^{4} p_i(\log p_i)$$

where 1.661 is a scaling constant and p_i is the proportional response to each of the n 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 (Smith and Travers 1979).

Each PbN cell was categorized as either a NAcc-projecting neuron if it was antidromically activated by NAcc stimulation or a non-NAcc-projecting neuron otherwise. Each PbN neuron was then further categorized either as a NAcc-suppressive neuron if stimulation of the NAcSh suppressed its spontaneous firing or as a non-NAcc-suppressive neuron if the NAcSh stimulation showed no effect. Therefore, a NAcc-projecting/suppressive neuron means that it sends its axon to the NAcc and also receives descending inhibitory input from the NAcc. Within these categories, each cell was classified as belonging to one of the four best stimulus categories based on their taste response profile. These categories were NaCl best, sucrose best, citric acid best, and QHCl best (Table 1).

Differences in mean firing rates between sucrose-sensitive and sucrose-insensitive neurons and among taste stimuli were compared using ANOVA. The effect of electrical stimulation of the NAcSh on spontaneous activity (duration of inhibition) and on gustatory response (reduction percentage) were also compared using ANOVA.

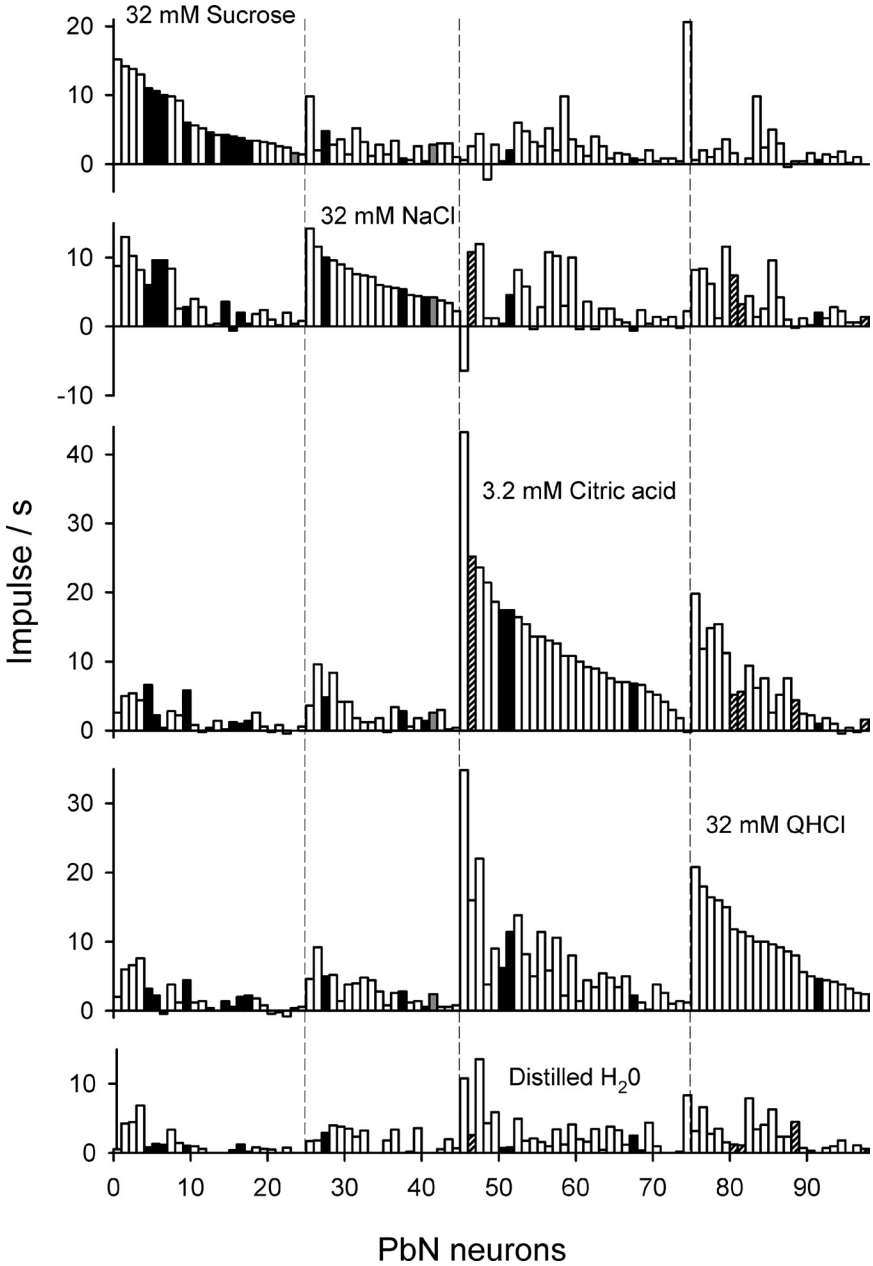

Fig. 3. Taste responses of 98 PbN neurons. Responses are net taste responses during the first 5 s of chemical stimulation [in impulses (imp)/s]. Cells are arranged along the abscissa according to their best stimulus, with *cells 1-25* being sucrose best, *cells 26-45* being NaCl best, *cells 46-75* being citric acid best, and *cells 76-98* being quinine hydrochloride (QHCl) best. Within each best stimulus group, cells are arranged according to the magnitude of the response to their best stimulus. The filled and dark gray bars indicate 16 ipsilateral NAcc-projecting cells and 2 contralateral NAcc-projecting cells, respectively. Hatched bars show the five PbN cells that responded neither antidromically nor orthodromically.

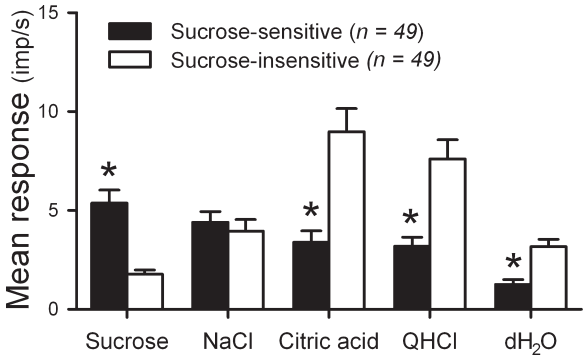

Fig. 4. Comparison of the mean firing rate (±SE) of PbN neurons in response to sucrose, NaCl, citric acid, QHCl, and distilled H_2O between the sucrose-sensitive and sucrose-insensitive groups.

Comparisons of spontaneous activities and individual gustatory responses between sucrose-sensitive and sucrose-insensitive groups were assessed using *t*-tests. Comparison of the number of neurons in each category was made via a χ^2-test. All means are reported with SEs unless otherwise stated.

RESULTS

Histology. A total of 98 taste-responsive PbN neurons were recorded from 29 male hamsters. The sites of the PbN neuron recorded last in each animal ($n = 29$) were examined histologically by the locations of Chicago sky blue dye markings. They were concentrated in the medial PbN, medial to the superior cerebellar peduncle, and dorsal and lateral to the mesencephalic trigeminal nucleus at the level of the mesencephalic trigeminal nucleus and locus coeruleus overlap. The anterior-posterior distribution of the recording marks extended rostrally from the level at which the locus coeruleus 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 previous recordings (Li and Cho 2006; Li et al. 2005; Mao et al. 2008). An example of a recording site marked with Chicago sky blue dye in the PbN is shown in Fig. 1*A*.

Stimulation sites in the NAcSh were also examined histologically. A representative brain section from the ipsilateral NAcSh is shown in Fig. 1*B*. A blood clot caused by the electrode damage to the wall of the lateral ventricle filled the site where the lower part of the electrode was positioned and a part of the ventral lateral ventricle. The tip of the electrode, marked by a blue iron deposit (arrow), was located in the NAcSh, medial to the anterior part of the anterior commissure. All stimulating sites from 29 animals were examined and reconstructed using standard atlas sections of the hamster brain (Morin and Wood 2001) (Fig. 2). The tips of the stimulating electrodes were confined to the NAcSh, medial to the core region of the NAcc, inferior and lateral to the intermediate part of the lateral septal nucleus, and inferior and slightly medial to the lateral ventricle. The stimulation sites that evoked antidromic activation and/or descending suppressive effects in the PbN (Fig. 2, filled circles) were intermingled with locations of those of no responses (Fig. 2, open circles).

Characteristics of the responses of taste neurons in the PbN. Each of the 98 neurons was categorized as either sucrose, NaCl, citric acid, or QHCl best on the basis of its response profile to the 4 basic taste stimuli. The numbers of sucrose-best, NaCl-best, citric acid-best, and QHCl-best cells were 25, 20, 30, and 23, respectively (Table 1). There was no significant bias in the distribution of taste-responsive PbN neurons sampled among the four best stimulus groups [$\chi^2 = 2.163$, degrees of freedom (df) = 3, $P = 0.539$]. The best stimulus categories for all PbN neurons are shown in Fig. 3. In Fig. 3, cells are grouped by the preferred taste category initially and then arranged along the abscissa in decreasing order by the magnitude of the response to the taste of the category they belong to. The response pattern of all 98 cells to a single taste is read horizontally and that of a neuron to all tastes is seen vertically. Each bar indicates the net response of a cell to a single taste. The last row shows the mean baseline activity (or spontaneous rate).

Forty-nine neurons responded to sucrose stimulation and were categorized as sucrose-sensitive cells, whereas the remaining forty-nine cells were classified as sucrose-insensitive cells (Fig. 4). As expected, overall taste responses between sucrose-sensitive and sucrose-insensitive groups were different [$F_{(1,384)} = 8.943$, $P < 0.005$]; in addition, responses to each of four taste stimuli were different from one another [$F_{(3,384)} = 5.565$, $P < 0.005$]. The interaction between sucrose sensitive-

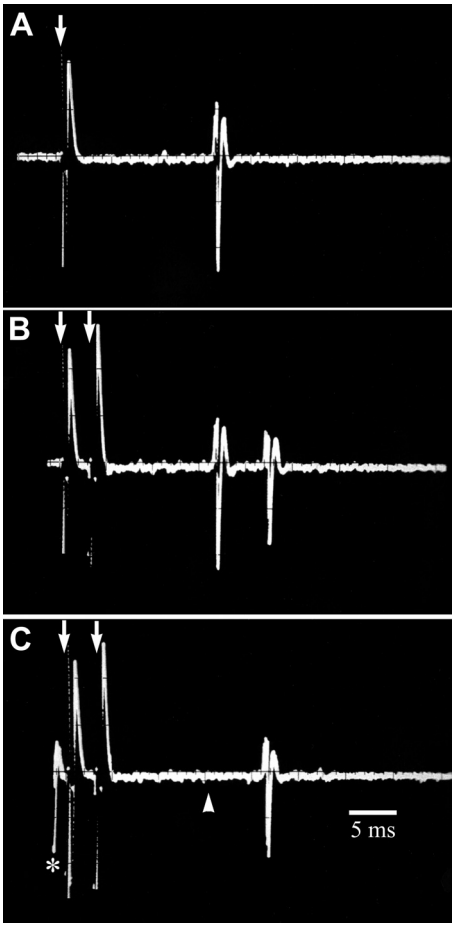

Fig. 5. Superimposed oscilloscope traces ($n \geq 3$ sweeps) recorded from a taste-responsive PbN cell showing the fulfillment of criteria for antidromic activation from the NAcSh. Ipsilateral NAcSh stimulation (arrow in *A*) evoked action potentials occurred at a constant latency (15 ms) and followed closely paired stimulus pulses (200 Hz; *B*). Collision between a spontaneously generated action potential (*) and a NAcSh stimulation-evoked spike (the first arrow in *C*) caused elimination of the evoked spike, which should have occurred at the arrowhead (*C*).

ness and stimulus was significant [$F_{(3,384)} = 18.424$, $P < 0.001$]; the response to sucrose (5.37 ± 0.66 impulses/s) was greater in the sucrose-sensitive group (1.77 ± 0.21 impulses/s, $t = 5.418$, df = 48, $P < 0.001$), whereas responses to citric acid ($t = 4.124$, df = 48, $P < 0.001$) and QHCl ($t = 4.514$, df = 48, $P < 0.001$) were significantly smaller. Baseline activities were also different ($t = 3.977$, df = 48, $P < 0.001$) between sucrose-sensitive (0–8.35 impulses/s, mean: 1.25 ± 0.25 impulses/s) and sucrose-insensitive PbN neurons (0.20–13.55 impulses/s, mean: 3.16 ± 0.37 impulses/s).

The entropy calculation showed that the NaCl-best neurons (mean: 0.86 ± 0.02, range: 0.36–0.98) were the most broadly tuned among the four best stimulus groups. The means of the other three groups were between 0.76 and 0.79. The difference between entropies among the four best stimulus groups was, however, not significant [$F_{(3,94)} = 2.278$, $P = 0.085$].

PbN taste neurons are antidromically activated by NAcSh stimulation. Eighteen of ninety-eight taste-responsive PbN cells (18.4%) were activated antidromically by stimulation of the NAcSh and were thus classified as NAcc-projecting neurons. Among them, the number of sucrose-best cells ($n = 10$, 55.6%) was significantly greater than the rest of the best stimulus groups ($\chi^2 = 10.003$, df = 3, $P < 0.05$; Table 1). The ipsilateral projection ($n = 16$, 88.9%) dominated over the contralateral projection ($\chi^2 = 10.889$, df = 1, $P < 0.005$); only two cells, a sucrose-best cell and a NaCl-best cell, projected to the contralateral NAcc. An example of a PbN neuron that demonstrated fulfillment of the criteria for antidromic activation is shown in Fig. 5. On average, the latency for antidromic activation was 6.09 ± 1.05 ms ($n = 18$, including 2 contralateral NAcc-projecting cells). The mean NAcSh stimulation threshold for antidromic activation was 30.61 ± 3.41 μA (range: 10–61 μA). A comparison of the latencies between ipsilateral- and contralateral-projecting cells was not attempted due to the smaller numbers of cells antidromically activated by contralateral NAcSh stimulation.

Effect of electrical stimulation of the NAcSh on spontaneous firing of PbN taste neurons. The effect of activation of descending input from the NAcSh on PbN neurons was examined by stimulating the NAcSh at a fixed intensity of 100 μA. We observed an effect of stimulation in the majority of PbN neurons (91 of 98 neurons, 92.9%), including 16 NAcc-projecting neurons. All of the observed responses were inhibitory. Examples of the inhibitory effect of NAcSh stimulation are shown in Fig. 6. PSTHs derived from two PbN neurons after stimulation of the ipsilateral and contralateral NAcSh are shown. As seen in the PSTHs, repeated single-pulse stimulation of the NAcSh at 1/3 Hz suppressed the ongoing firing activity of PbN neurons immediately after the onset of the stimulation (0 ms), implying that NAcSh activation inhibits the neuronal activity of PbN taste-responsive neurons.

The majority of PbN taste neurons (88 of 98 cells, 89.8%) were inhibited by ipsilateral NAcSh stimulation ($\chi^2 = 62.082$, df = 1, $P < 0.001$). Among the 10 neurons unresponsive to ipsilateral NAcSh stimulation, QHCl-best neurons were dominant ($n = 7$, $\chi^2 = 12.087$, df = 3, $P < 0.01$). The number of PbN neurons inhibited by contralateral NAcSh stimulation ($n = 55$) was not significantly different from that of non-NAcSh-suppressed neurons ($\chi^2 = 1.469$, df = 1, $P = 0.225$). Therefore, the descending inhibitory input to gustatory PbN neurons from the ipsilateral NAcSh seems stronger than that from the contralateral NAcSh ($\chi^2 = 7.615$, df = 1, $P < 0.01$). Fifty-two PbN gustatory neurons were inhibited bilaterally: fourteen sucrose-best cells, thirteen NaCl-best cells, fourteen citric acid-best cells, and eleven QHCl-best cells. The neurons that did not respond to either side of NAcSh stimulation included four QHCl-best neurons, one sucrose-best neuron, one NaCl-best neuron, and one citric acid-best neuron (see Table 1). We measured the durations during which the spontaneous activities were suppressed. The mean duration of inhibition was comparable between ipsilateral (71.22 ± 2.86 ms, range: 26–137 ms) and contralateral (67.75 ± 3.38 ms, range: 29–123 ms) NAcSh stimulation [$F_{(1,142)} = 0.596$, $P = 0.442$].

Effect of electrical stimulation of the NAcSh on taste responses of PbN neurons. The effect of electrical stimulation of the NAcSh on the spontaneous activity of PbN neurons was exclusively inhibitory. To test whether the descending input from the NAcc exerts the same inhibitory influence on gustatory responses, we compared taste responses of PbN cells with or without electrical stimulation of the NAcSh. We used two stimulating methods depending on the firing characteristics of

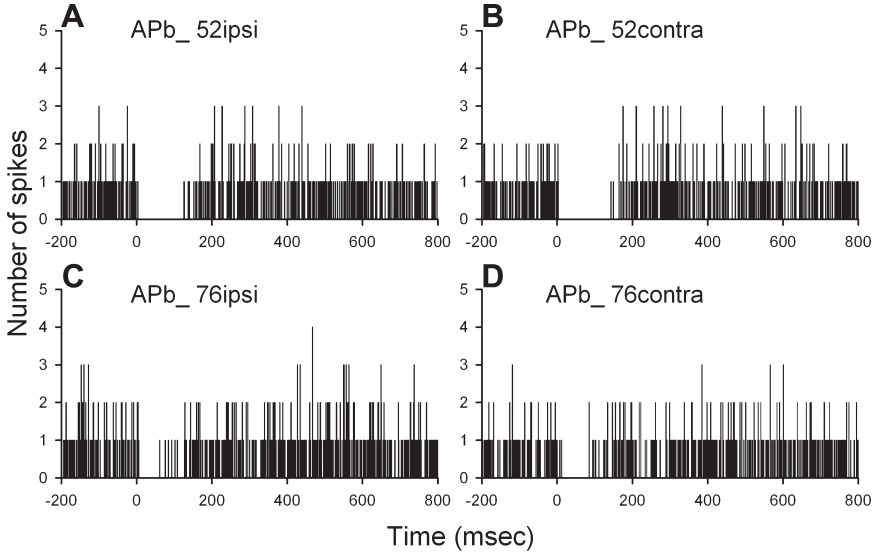

Fig. 6. Peristimulus time histograms (PSTHs) showing the responses of two PbN taste-responsive neurons after single-pulse electrical stimulation (ES) of the ipsilateral (*A* and *C*) or contralateral (*B* and *D*) NAcSh. ES of the NAcSh produced inhibitory responses in these two cells. Electrical pulses were delivered to the NAcSh at *time 0*; PSTHs were accumulated over 200 stimulus sweeps.

the PbN taste neurons: PbN neurons spontaneously active and those spontaneously less active or inactive. For spontaneously active PbN gustatory neurons, taste trials were repeated during NAcSh stimulation, and taste responses derived from these experimental schemes were compared with original taste responses without NAcSh stimulation (see METHODS). An example of the effect of NAcSh stimulation on taste responses of a PbN neuron, which responded best to NaCl, is shown in Fig. 7*A*. The gustatory response to NaCl was reduced during stimulation to either NAcSh (reduced to 53.52% and 50.70% of the original response with ipsilateral or contralateral stimulation, respectively). Taste responses were recovered to the original level 15 min after the termination of NAcSh stimulation. The same analysis was carried out in 16 of 91 orthodromically responsive PbN cells, including 10 neurons that were inhibited bilaterally and 6 neurons that were inhibited ipsilaterally. Suppression of gustatory responses during NAcSh stimulation was observed for all neurons tested. The amount of reduction ranged from 21.74% to 78.00% (mean: 53.58 ± 1.14%) and was significant for both ipsilateral [$F_{(2,135)} = 29.665$, $P < 0.0001$] and contralateral [$F_{(2,72)} = 18.572$, $P < 0.0001$] NAcSh stimulation.

There was no significant difference in the response reduction across the four tastes by electrical stimulation of either the ipsilateral [$F_{(3,135)} = 0.496$, $P = 0.686$] or contralateral [$F_{(3,72)} = 0.640$, $P = 0.592$] NAcSh. The reductions were not

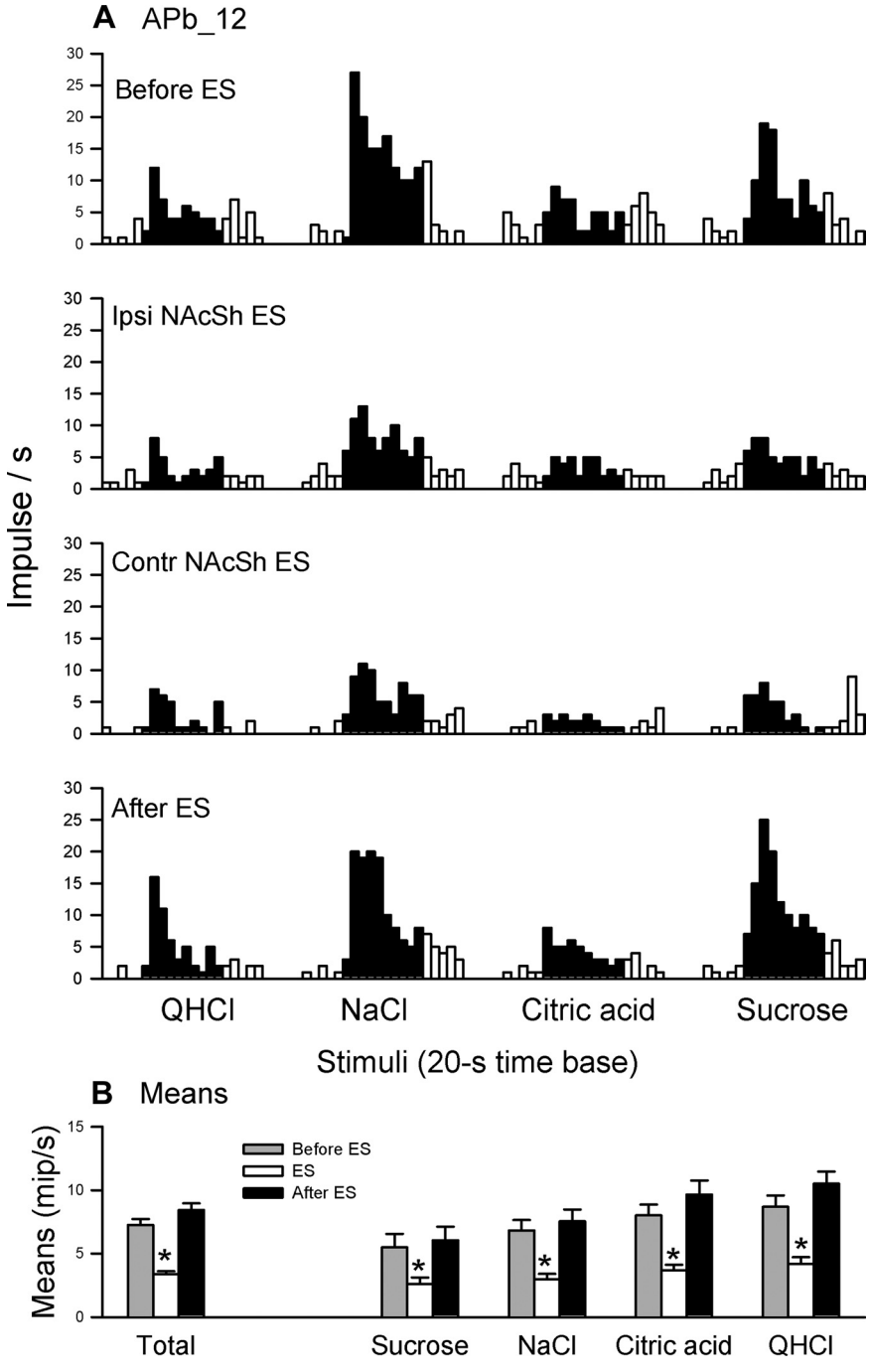

Fig. 7. *A*: taste response profiles of a NaCl-best PbN neuron to taste stimulation before and during ES of the NAcSh. Each histogram reflects 20 s of cell activity. Control responses to each of the four taste stimuli are shown at the *top*. The same taste trials, repeated during ES of the ipsilateral or contralateral NAcSh, are shown in the *middle*. The taste responses recovered 15 ms after the taste trials with the NAcSh stimulation are shown at the *bottom*. *B*: mean firing rates to all taste stimuli (total) and to each of the four basic taste stimulus before, during, and after ES of the NAcSh. Responses to all taste stimuli and to each of the four taste stimuli were significantly decreased by NAcSh stimulation.

significantly different among the four taste stimuli [$F_{(3,73)}$ = 0.540, P = 0.656, range from 51.73 ± 2.69% (QHCl) to 55.83 ± 1.52% (NaCl)]. There was no significant difference between the effect of ipsilateral (54.81 ± 1.17%) and contralateral (51.45 ± 2.37%) NAcSh stimulation on the taste response [$F_{(1,69)}$ = 2.810, P = 0.098]. Recovery of the taste responses was examined by repeating the same taste trials 15 min later without NAcSh stimulation. The inhibitory effects by NAcSh stimulation were abolished in all the neurons tested. In fact, the magnitudes of gustatory responses were slightly greater than those measured before NAcSh stimulation [$F_{(1,152)}$ = 4.112, P = 0.044]. The overall effect of NAcSh stimulation on the gustatory response is shown in Fig. 7*B*.

For PbN taste-responsive neurons with low spontaneous firing (<0.2 impulses/s), the effect of descending NAcSh input on the taste response was examined by applying a single pulse shock to the NAcSh (0.1 mA, 1/3 Hz, 0.5 ms) while elevating the firing of PbN cells by taste stimulation. The concentration of the taste solutions used to elicit taste responses were adjusted so as to activate the neuron at a moderate firing rate (see METHODS for details). Seventeen PbN cells were tested using this experimental protocol. For the first five cells tested using this experimental scheme, individual taste solutions were used separately in addition to a mixture of effective tastes. The results from these five cells showed that NAcSh activation suppressed taste responses regardless of whether they were induced by an individual taste or a mixture of tastes. Thus, for the rest of the 12 cells tested, only the mixture of tastes was used. NAcSh stimulation inhibited taste mixture-induced firing of all 12 cells tested, as shown in Fig. 8.

DISCUSSION

In the present study, we investigated the functional relationships between the NAcSh and gustatory PbN. Our main finding was that the majority of pontine taste-responsive neurons are under the modulatory influence of the bilateral NAcSh and that this descending input from the NAcSh exerts an exclusively inhibitory effect. In addition, our work showed that a subset of pontine gustatory neurons, mostly sucrose-best cells, project to the NAcc. These data provide straightforward evidence for the presence of reciprocal neural connections between the NAcSh and gustatory PbN and demonstrate that taste processing in the PbN could be modulated by the activation of the NAcSh.

Neural connection between gustatory PbN and the NAcSh. Sweetness is innately preferred by rodents and humans alike (Danilova et al. 1998; Johanson and Hall 1979; Steiner 1979). Sucrose intake induces dopamine release in the NAcSh, regardless of whether the feeding is real or sham (Hajnal et al. 2004; Norgren et al. 2006; Smith 2004). With sham feeding there is no postprandial effect of sucrose, since it is blocked from entering the intestine. Thus, the dopamine release induced by sham feeding must be triggered by orosensory cues. The dopamine release in the NAcSh does not simply reflect the hedonic value of food. Dopamine is released in the NAcSh only when the animal has preexposure to the appetitive stimulus of food (Bassareo and Di Chiara 1999).

The medial PbN is the second taste relay nucleus in the taste pathway that receives gustatory information from the NST (Lundy and Norgren 2004b; Norgren and Leonard 1971; Norgren and Pfaffmann 1975). Lesions of the PbN, but not the VPM, block conditioned taste aversion or sodium appetite (Dayawansa et al. 2011; Grigson et al. 1997, 1998; Hajnal and Norgren 2005; Mungarndee et al. 2006). Similarly the PbN, but not the VPM, is necessary for sucrose-induced dopamine release in the NAcSh (Hajnal and Norgren 2005; Hajnal et al. 2004; Norgren et al. 2006). Increased c-*fos* expression in the ventral forebrain gustatory nuclei and NAcSh after sucrose sham feeding is abolished by lesions of the medial (gustatory) but not lateral PbN (Mungarndee et al. 2008). These studies suggest there is neural communication between the NAcSh and gustatory PbN. The NAcSh may receive the information about the hedonic value of foods from the gustatory nuclei in the brain stem directly or indirectly. If taste information is transmitted to the NAcc from the brain stem, bypassing the ventral forebrain, the PbN is a likely candidate. The PbN sends axons to various nuclei in the ventral and limbic brain (Lundy and Norgren 2004b; Moga and Gray 1985; Norgren 1974, 1976) and receives descending projections from them, including the NAcSh (Usuda et al. 1998).

In the present study, we checked for the presence of a direct projection from the PbN to the NAcSh using antidromic stimulation. Eighteen of ninety-eight PbN neurons (18%) were

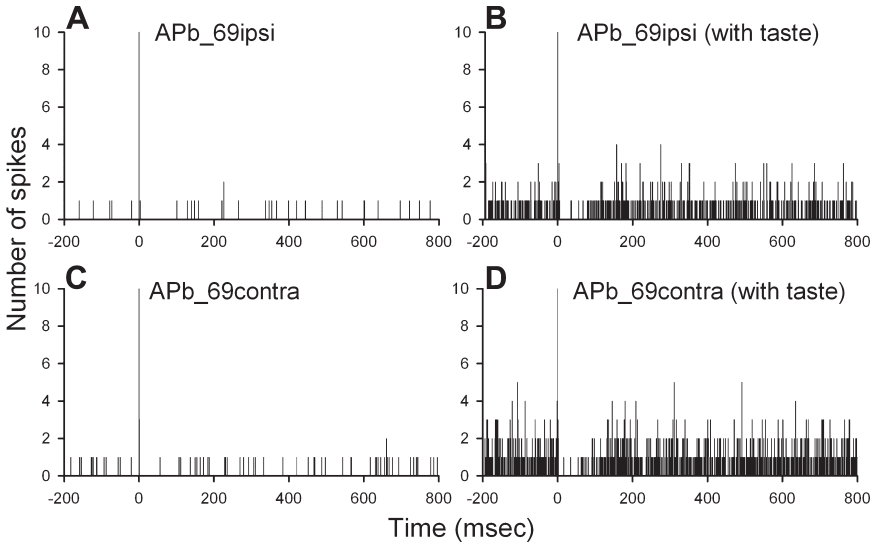

Fig. 8. PSTHs showing the effect of NAcSh stimulation on the firing activity of a PbN neuron created without (*A* and *C*) or with (*B* and *D*) chemical stimulation of the tongue. When we delivered 200 stimulus pulses to the ipsilateral NAcSh (*A*) or contralateral NAcSh (*C*), we could not observe suppression of the cell activity due to the low spontaneous firing activity. NAcSh-induced inhibition of the same cell was evident when the firing activity was elevated by stimulation of the anterior tongue using a taste mixture (*B* and *D*). ES started at *time 0*. Each PSTH was accumulated over 200 stimulus sweeps. The peak in each PSTH at *time 0* represents a stimulus artifact.

antidromically driven, sixteen of which projected to the ipsilateral NAcSh (16%). In a previous study, we observed that 13 of 117 (10%) pontine taste neurons, including one projecting to the contralateral BNST, were antidromically activated in response to electrical stimulation of the BNST (Li and Cho 2006). Anatomically, the BNST is immediately posterior to the NAcc, raising the concern that the antidromic responses observed in the present study were seen because the NAcSh stimulating current might have spread to the BNST. The distance between these two sites, calculated from the coordinates we used in our experiments, is ~1.85 mm. It is known that a current of 100 μA activates all axons contained within a sphere of 60-μm radius, and 50% of the axons located within 108-μm radius, from the stimulating electrode (Nowak and Bullier 1996). Although the current we used to determine antidromic status was as high as 100 μA, the mean threshold for antidromic activation of the PbN cells was much smaller (mean: 30.61 $\pm$ 3.41μA, range: 10–61 μA). Thus, we believe that the chance of the current delivered from the NAcSh spreading to the BNST was minimal.

Inhibitory modulation of taste responses in the PbN by descending projection from the NAcSh. One of the most important findings of this study is that gustatory PbN neurons are subject to a modulatory influence from the NAcSh. The descending influence from the NAcSh to gustatory PbN cells was characterized by its extensiveness (88 of 98 cells, 90%) and by its exclusively inhibitory nature. In previous electrophysiological studies, we demonstrated that gustatory neurons in the hamster brain stem are under descending influences from the VPM, gustatory cortex, and various forebrain nuclei, including the LH, CeA, and BNST (Cho and Li 2008; Cho et al. 2002a, 2002b, 2003, 2008; Li and Cho 2006; Li et al. 2002, 2005, 2008; Mao et al. 2008). The influences from these forebrain nuclei to gustatory cells in the PbN and NST showed different characteristics. Overall, more PbN neurons responded both antidromically and orthodromically to electrical stimulation of ventral forebrain nuclei than neurons in the NST. These observations suggest the connections between the ventral forebrain nuclei and gustatory PbN are more extensive than to the gustatory NST (Cho et al. 2002b, 2008; Li et al. 2002, 2005; Mao et al. 2008). The present study showed that the descending input from the NAcSh exerts an exclusively inhibitory influence on activity of taste-responsive cells in the PbN. Similarly, descending inputs from the BNST and VPM to gustatory PbN cells were also mostly inhibitory, and activation of the BNST and VPM suppressed gustatory responses of the PbN cells (Li and Cho 2006; Mao et al. 2008). To investigate whether NAcSh activation modulated gustatory responses, we compared taste responses with/without electrical stimulation of the NAcSh. Indeed, electrical stimulation of the NAcSh suppressed gustatory responses in all the neurons tested; e.g., high-frequency electrical stimulation of the NAcSh reduced the firing of PbN cells to gustatory stimulation by 54%. As in previous studies, electrical stimulation of the NAcSh suppressed gustatory responses, regardless of which type of taste stimuli. Because activation of the descending projections from the NAcSh modulate responses of pontine gustatory neurons in response to a palatable stimulus (sucrose) as well as to an aversive stimulus (QHCl), it is not likely that NAcSh activation could preferentially target palatable over aversive taste processing. Since the majority of the gustatory cells in the PbN project to the VPM (Mao et al. 2008) as well as to ventral forebrain targets (Li et al. 2005), it seems likely that NAcSh activation reduces the signal-to-noise ratio in taste information processing.

Physiological implications. Physiological factors associated with nutritional homeostasis, such as blood insulin and glucose levels, gastric distention, sodium appetite, and taste aversion, can alter taste-evoked neuronal activities of some PbN neurons (Baird et al. 2001; Cho et al. 2004; Shimura et al. 1997; Tamura and Norgren 1997). These studies suggest that homeostatic information may affect gustatory processing in the PbN. In previous studies, we demonstrated that gustatory processing in the PbN is under the influence of the various forebrain nuclei that are also involved in maintaining nutritional homeostasis.

Whether palatable or aversive stimuli activate NAcSh neurons was not directly addressed in the present study. However, our data suggest that palatable taste information may preferentially activate NAcSh cells because most of the neurons (10 of 18 neurons) shown to project to the NAcSh were sucrose-best cells. Whether some NAcSh neurons are activated or inactivated by the hedonic value of orosensory stimuli or by motivated behavior, and/or whether a palatable stimulus exerts a differential influence on NAcSh neurons, requires further investigation. The present data verified that spontaneous and/or taste-evoked activity of the majority of gustatory PbN neurons was modulated by activation of the NAcSh. Since neurons in the NAcSh track the reward value of stimuli, this descending projection may be involved in modulation of the motivational value of pontine gustatory processing.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.-S.L. and Y.K.C. conception and design of research; C.-S.L. and Y.K.C. performed experiments; C.-S.L., S.C., D.-P.L., and Y.K.C. analyzed data; C.-S.L. and Y.K.C. interpreted results of experiments; C.-S.L. and Y.K.C. drafted manuscript; C.-S.L., S.C., and Y.K.C. edited and revised manuscript; C.-S.L., S.C., D.-P.L., and Y.K.C. approved final version of manuscript; D.-P.L. and Y.K.C. prepared figures.

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