Characteristics of Rostral Solitary Tract Nucleus Neurons With Identified Afferent Connections That Project to the Parabrachial Nucleus in Rats

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Suwabe T, Bradley RM. Characteristics of rostral solitary tract nucleus neurons with identified afferent connections that project to the parabrachial nucleus in rats. J Neurophysiol 102: 546–555, 2009. First published May 13, 2009; doi:10.1152/jn.91182.2008. Afferent information derived from oral chemoreceptors is transmitted to second-order neurons in the rostral solitary tract nucleus (rNST) and then relayed to other CNS locations responsible for complex sensory and motor behaviors. Here we investigate the characteristics of rNST neurons sending information rostrally to the parabrachial nucleus (PBN). Afferent connections to these rNST-PBN projection neurons were identified by anterograde labeling of the chorda tympani (CT), glossopharyngeal (IX), and lingual (LV) nerves. We used voltage- and current-clamp recordings in brain slices to characterize the expression of both the transient A-type potassium current, $I_{\text{KA}}$, and the hyperpolarization-activated inward current, $I_{\text{h}}$, important determinants of neuronal repetitive discharge characteristics. The majority of rNST-PBN neurons express $I_{\text{KA}}$, and these $I_{\text{KA}}$-expressing neurons predominate in CT and IX terminal fields but were expressed in approximately half of the neurons in the LV field. rNST-PBN neurons expressing $I_{\text{h}}$ were evenly distributed among CT, IX and LV terminal fields. However, expression patterns of $I_{\text{KA}}$ and $I_{\text{h}}$ differed among CT, IX, and LV fields. $I_{\text{KA}}$-expressing neurons frequently coexpress $I_{\text{h}}$ in CT and IX terminal fields, whereas neurons in LV terminal field often express only $I_{\text{h}}$. After GABA$_A$ receptor block all rNST-PBN neurons responded to afferent stimulation with all-or-none excitatory synaptic responses. rNST-PBN neurons had either multipolar or elongate morphologies and were distributed throughout the rNST, but multipolar neurons were more often encountered in CT and IX terminal fields. No correlation was found between the biophysical and morphological characteristics of the rNST-PBN projection neurons in each terminal field.

INTRODUCTION

The rostral nucleus of the solitary tract (rNST) is the first central relay in the taste pathway (reviewed in Bradley 2006). Sensory information originating from taste buds and other receptors in the oral cavity is relayed to the CNS via afferent nerve fibers of the facial and glossopharyngeal nerves. The central processes of these nerves form the solitary tract (ST), which travels caudally in the brain stem giving off branches that terminate within the rNST (Hamilton and Norgren 1984; May and Hill 2006). The chorda tympani (CT) and greater superficial petrosal branches of the facial nerve innervate the anterior tongue and palate, respectively, and project to the most rostral portions of the nucleus. The terminals of these branches of the facial nerve intermingle within a region of the dorsal rNST just medial to the ST; an area corresponding to rostral-central and medial rostral-lateral subdivisions (Whitehead 1988). The lingual-tonsils branch of the glossopharyngeal nerve (IX) transmits sensory information from the posterior tongue to a region of the rNST that overlaps the caudal part of the facial nerve terminal field and extends into the caudal rNST (Halsell et al. 1993). The IX nerve has two distinct terminal fields termed T1 and T2 (Lasiter 1992). The T2 field corresponds to the rostral-central subdivision of the rNST, and neurons in this field receive both taste and tactile input from the IX nerve. The lingual branch of the trigeminal nerve (LV) transmits tactile information from the oral epithelium to the rNST and overlaps with the T1 terminal field of the IX nerve, terminating primarily within the rostral-lateral subdivision of the rNST (Halsell et al. 1993; Spector and Travers 2005; Travers 1993).

Sensory information relayed by these afferent nerves synapses with second-order neurons in rNST. As the first central structure to receive orosensory input, one of the main roles of the rNST is to disseminate this information to brain areas involved in sensory perception and the generation of sensory related behavioral and physiological responses. Accordingly, neural pathways originating in the rNST ascend to higher brain centers and descend to medullary oromotor nuclei (reviewed in Lundy and Norgren 2004).

In rodents and lagomorphs, the ascending pathway includes an obligatory synapse in the caudal parabrachial nucleus (PBN) in the pons (Herbert et al. 1990; Krukoff and Scott 1984; Norgren and Leonard 1971; Travers 1988). Neurons responding to orosensation are concentrated in the “waist” area of the PBN that includes the central medial and ventral lateral subnuclei as well as neurons that bridge the brachium conjunctivum (Halsell and Travers 1997; Norgren and Pfaffmann 1975). This projection arises from multipolar and elongate rNST neurons mainly located within the rostral central subdivision of rNST (Gill et al. 1999; Halsell et al. 1996; Whitehead 1990). About 60% of the neurons in the rostral central subdivision can be retrogradely labeled following injection of a neural tracer into the caudal PBN (Whitehead 1990), implying that a major role of the rNST is to convey gustatory information to the PBN, and estimates from electrophysiological data indicate that between 31 and 80% of taste-responsive rNST neurons project to the PBN (Cho et al. 2002; McPheeters et al. 1990; Monroe and Di Lorenzo 1995). The remaining rNST neurons although responding to chemical stimulation of the tongue have no role in taste perception functioning as the input limb of taste generated oral reflexes such as initiation of salivary secretion and other oral-motor reflexes (reviewed in Bradley and Kim 2006). Thus separate populations of rNST neurons...
have different roles in processing chemosensory information resulting from stimulation of taste buds.

Because the intrinsic firing properties of neurons control the transformation of input into action potential outputs, the present study is focused on characterization of the properties of the rNST-PBN projecting neurons that process gustatory information involved in taste perception. Investigators have reported that rNST neurons express voltage-gated currents important in the control of repetitive firing properties. In “blind” brain slice recordings, the hyperpolarization-activated potassium current—\( I_{K_A} \)—has been shown to be expressed in rNST neurons (Tell and Bradley 1994; Uteshev and Smith 2006). A second hyperpolarization-activated current (\( I_h \)) shown to be present in rNST is also an important modulator of action potential firing frequency (Tell and Bradley 1994; Uteshev and Smith 2006). Thus we explored \( I_{K_A} \) and \( I_h \) expression in identified rNST-PBN projection neurons in voltage- and current-clamp recordings.

To identify rNST-PBN projecting neurons, we used retrograde fluorescent labeling of PBN-projecting rNST neurons. In addition, we have combined the retrograde labeling with anterograde fluorescent tracing of the terminal fields of afferent gustatory nerves to explore the electrophysiological properties of rNST-PBN projection neurons with defined afferent input from peripheral oral receptors.

**METHODS**

**Retrograde labeling of rNST-PBN-projecting neurons**

Adult male Sprague-Dawley rats (260–320 g, ~60–80 days old) were used in this study. All surgical procedures were carried out under National Institutes of Health and University of Michigan Animal Care and Use Committee approved protocols. Rats were anesthetized with intraperitoneal injection of a mixture of ketamine (80 mg/kg) and xylazine (16 mg/kg) and placed in a stereotaxic instrument (Stoelting) with atraumatic ear bars. rNST-PBN projecting neurons were identified by retrograde labeling from the PBN using published coordinates (Halsell et al. 1996; Paxinos and Watson 1998; Williams et al. 1996). Anatomical coordinates for successful injections were 8.8–9.2 mm caudal to bregma, 1.6–1.8 mm lateral to the midline, and 7.0–7.3 mm ventral to the skull surface. The retrograde tracer 1,1-diododecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, Invitrogen) was iontophoresed into the PBN (Fig. 1A) to retrogradely label the rNST-PBN projection neurons. The injection pipettes (8–20 μm ID, <35 μm OD) were fabricated from borosilicate glass capillaries (BF-120-60-10, Sutter Instrument) with a Flaming-Brown micropipette puller (P-80/PC, Sutter Instrument) and filled with 0.5% DiI dissolved in ethanol. The DiI was delivered by positive current pulses (0.8 μA for 3 min of 10 s on and 10 s off) generated by a square pulse stimulator (S48, Grass Technologies) and a stimulus isolation unit (PSIU6, Grass Technologies).

**Anterograde labeling of afferent terminal fields**

Five days after labeling the PBN the rats were reanesthetized, and the terminal field of either the CT, lingual-tonsillar branch IX or lingual branch of LV nerves was anterogradely labeled (Brining and Smith 1996; Contreras et al. 1980; May and Hill 2006). The CT or LV was exposed by a ventral approach, and the CT nerve was cut in the tympanic bulla. The LV nerve was cut just distal to the junction with the CT nerve. The proximal cut ends were placed on Parafilm and crystals of a green fluorescent tracer (Alexa Fluor 488 dextran, Molecular Probes) applied to the proximal cut end and sealed in place.
with silicone glue (Kwik-Cast, World Precision Instruments) to prevent dye spread.

Previous studies have reported that IX terminal field consists of two separate regions termed T1 and T2 (Lasiter 1992). We therefore double labeled the LV and IX terminal fields to ensure that recordings from the rNST-PBN projection neurons were contained in the T2 terminal field area exclusively innervated by the IX nerve. The IX nerve was exposed medial to the tympanic bulla and cut near the petrosal ganglion. The proximal cut end was placed on Parafilm and crystals of Alexa Fluor 488 dextran applied to the proximal cut end and sealed with silicone glue. The LV terminal field was labeled as described in the preceding text except using a red fluorescent tracer (Alexa Fluor 568 dextran). Surgical incisions were sutured closed and the rats maintained on a heating pad until ambulatory. Survival time for anterograde labeling of nerve terminal fields was 18 h to 2 days.

**Preparation of brain stem slices**

After time for anterograde transport, the rats were anesthetized with halothane and decapitated, and the brain was rapidly removed and cooled for 5–8 min in an oxygenated physiological saline solution in which NaCl was replaced with isosmotic sucrose at 4°C (Aghajanian and Rasmussen 1989). The brain stem was transected at the level of the pons and just caudal to the obex and cemented to a Vibratome (Technical Products International) stage with cyanoacrylate glue. The brain stem was sectioned horizontally into 250-μm-thick slices. Slices were incubated for ≥1 h in an oxygenated artificial cerebrospinal fluid (ACSF) at room temperature before transferring to a recording chamber. ACSF contained (in mM) 124 NaCl, 5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 26 NaHCO₃, 1.25 NaH₂PO₄, and 25 dextrose, was gassed with a 95% O₂-5% CO₂ mixture to achieve a solution pH of 7.4.

**Recording**

Slices were transferred to a recording chamber attached to the stage of a microscope (ECLIPSE E600-FN, Nikon) and anchored with a nylon mesh. During recording, the slice was perfused at 2–2.5 ml/min with oxygenated ACSF at 32°C. Retrogradely labeled rNST-PBN neurons and anterogradely labeled afferent terminal fields were identified using epifluorescent illumination. The identified neurons were observed using infrared-differential interface contrast optics (IR-DIC) via a CCD camera (IR-1000, DAGE-MTI). The identified neurons were recorded in whole cell mode using a patch-clamp amplifier (Axoclamp-2B, Axon Instruments). Signals were recorded through 2-KHz low-pass filter, digitized at 20–50 kHz (DigiData 1200, Axon Instruments), and stored on a computer hard disk. Data were acquired using pCLAMP 8 (Axon Instruments). Patch pipettes were made of borosilicate glass capillaries (TW150F-4, World Precision Instruments) using a two-stage puller (PP-83, Narishige) and filled with a solution that contained (in mM) 130 K-glucone, 10 n-2-hydroxy-ethylpiperazine- N’-2 ethane-sulfonic acid (HEPES), 10 ethylene glycol-bis(β-aminoethyl ether)- N,N,N’,N’-tetraacetic acid (EGTA), 1 MgCl₂, 1 CaCl₂, and 2 ATP, buffered to pH 7.2 with KOH. Lucifer yellow (Sigma) was dissolved in the pipette solution at a concentration of 0.1% to label recorded neurons. Tip resistance of filled pipettes was 6–8 MΩ.

**Postsynaptic current (PSC)**

To evoke PSCs in rNST-PBN projection neurons, a bipolar electrode (125 μM OD: FHC) was carefully positioned on the ST and current stimuli (<500 μA, 0.2-ms duration) delivered by a square pulse stimulator (S88, Grass Technologies). The stimulation site was separated from the recording site by ≥1 mm. To isolate the excitatory component of the postsynaptic currents (EPSC), the inhibitory components (Grabauskas and Bradley 1996; Wang and Bradley 1995) was eliminated by either clamping the neurons at −70 mV or by superfusing the GABAₐ receptor antagonist, bicuculline (10 μM) over the slices. Synaptic latency of the PSC s was measured from the onset of the ST stimulation to the time the evoked PSC exceeded the noise level. Jitter was calculated for each neuron as the SD of the latency of repeatedly elicited PSCs (>20).

**Neuron reconstruction**

After patch-clamp recording, slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 24 h. Fixed slices were rinsed in the phosphate buffer for 30 min and embedded in 4% agar, then cut into 100-μm-thick sections on a Vibratome. The sections were mounted on glass slides, dried overnight, and fitted with a coverslip with 80% triple mounting medium (ProLong Gold, Molecular Probes).

Neurons filled with Lucifer yellow were scanned and serial image stacks captured every 1 μm using a confocal microscope (MRC-600, Bio-Rad). Filled neurons were reconstructed from the stacked confocal images using neuroanatomical analysis software (Neurolucida, MBF Bioscience).

**Analysis of ionic currents**

Expression of voltage-gated currents and repetitive discharge patterns in rNST-PBN projection neurons were examined using voltage-clamp and current injection protocols. Voltage dependence of the activation of I₁,KA was assessed by holding the membrane at −110 mV for 800 ms and then depolarizing the membrane with 1,200-ms long steps, to voltages ranging from −20 to 0 mV (Figs. 3F and 4H). In voltage-clamp recordings, I₁,KA was isolated using tetraethylammonium (TEA, 10 mM) and 4-aminopyridine (4-AP, 1 mM; both obtained from Sigma-RBI) superfused over the slices (Fig. 3B and C). I₆ was identified in current clamp recordings by the presence of voltage “sag” when the membrane was hyperpolarized greater than −100 mV by current injection (Fig. 5A). I₆ currents were examined in voltage-clamp recordings using a series of hyperpolarizing voltage steps (~20 mV, 800-ms duration) from a holding potential of −60 to −120 mV (Fig. 5B, bottom). The rapid transient inward current was concurrent with the voltage sag response to hyperpolarizing current injections in current-clamp recordings. Both the voltage sag and inward current was blocked by 3 mM CsCl (Fig. 5C) superfused over the slices.

**Data analysis**

Electrophysiological data were analyzed using the Clampfit program (Axon Instrument). The junction potential due to potassium gluconate (10 mM) was subtracted from the membrane potential values. Statistical analysis was conducted using the SPSS program (SPSS). The numerical values are given as means ± SE, and statistical significance (P < 0.05) was assessed using Student’s t-test or ANOVA with a post hoc test for comparison of mean values and χ² test or Fisher’s exact test for comparison of proportion.

**Histology**

At the end of each experiment, the pontine area was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for ≥24 h and then cut into 100- to 200-μm-thick coronal sections. The sections were mounted on glass slides and dried overnight. Brightfield images of the PBN and surrounding tissue were recorded using a fluorescence microscope equipped with DIC optics (Eclipse 80i, Nikon). Locations of the injection sites were mapped on standard diagrams prepared from Nissl-stained sections of the rat brain.

**Results**

**PBN injection sites**

Examination of the PBN sections indicated that the injection site was typically limited to the region surrounding the
waist area. Figure 1A shows a representative DiI-injection site. Successful tracer injections labeled projection neurons throughout the rNST (Fig. 1B). The size of the DiI injection site was similar in all the animals and ranged from 200 to 300 μm in maximal diameter. The centers of the injection sites were concentrated within and adjacent to the waist area (Fig. 1, C–F).

Electrophysiological properties of the rNST-PBN projection neurons

The results of this study are based on recordings from a total of 101 retrogradely labeled rNST-PBN projection neurons. Of these neurons 38, 28, and 35 were recorded in the antero-gradely labeled CT, LV, and IX nerve terminal fields, respectively (Figs. 2, A–C). The mean values of resting membrane potential and input resistance of all the recorded neurons as well as neurons in each labeled terminal field are summarized in Table 1. The mean membrane potential of rNST-PBN neurons in the IX terminal field was significantly more negative than neurons in LV terminal field area (Bonferroni post hoc test: \( P < 0.01 \)). In addition, the input resistance of rNST-PBN neurons in the IX terminal field was significantly higher than neurons in the CT as well as LV terminal fields (Bonferroni post hoc test: \( P < 0.01 \)).

Expression of \( I_{KA} \) by rNST-PBN projecting neurons

The majority of the rNST-PBN-projection neurons \( (n = 73 \) or 72%) expressed \( I_{KA} \) (Fig. 3A). The amplitude of the transient current varied widely from 57 to 1031 pA as measured by the difference between the early transient and steady-state outward currents (● – ○ in Fig. 3A). We used pharmacological blockers in a subset of rNST-PBN neurons \( (n = 12) \) to confirm the identity of the transient current. The steady-state outward current was blocked by bath application of a potassium channel blocker, tetraethylammonium chloride, (TEA, 10 mM; compare Fig. 3, A with B). The early transient outward current remained during application of TEA alone (Fig. 3B) and was eliminated after 1 mM 4-AP was added to the superfusate (compare Fig. 3, B with C). These results confirm that the transient outward current results from activation of \( I_{KA} \) (Tell

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**Table 1. Biophysical properties of rNST-PBN projection neurons in the CT, LV, and IX terminal fields**

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<th></th>
<th>CT</th>
<th>LV</th>
<th>IX</th>
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<td>( V_{rest}, \text{mV} )</td>
<td>(-53 \pm 1 )</td>
<td>(-50 \pm 1^{IX} )</td>
<td>(-57 \pm 1^{LV} )</td>
</tr>
<tr>
<td>( R_{input}, \text{MΩ} )</td>
<td>(338 \pm 27^{LV} )</td>
<td>(362 \pm 28^{IX} )</td>
<td>(495 \pm 31^{CT,LV} )</td>
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\( V_{rest} \): resting membrane potential; \( R_{input} \): input resistance. Values of \( V_{rest} \) and \( R_{input} \) are means ± SE. Statistical significance differences at \(<0.05 \) level versus neurons recorded in chorda tympani \( (CT^{CT}) \), lingual \( (LV^{LV}) \), and glossopharyngeal \( (IX^{IX}) \) terminal fields.

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**Fig. 2.** rNST-PBN-projection neurons in the anterogradely labeled terminal fields of the CT (A), IX (B), and LV (C) nerves. The 3 photomicrographs in C are serial sections from the same specimen. Top section is more ventral than the lower 2 sections. The rNST-PBN projection neurons appear yellow due to merging of the green (terminal field) and red (retrogradely labeled neuron) images. ISN, retrogradely labeled neurons of the inferior salivatory nucleus. Asterisks indicate ST. Scale bars = 100 μm.

**Fig. 3.** Two types of voltage-dependent outward currents recorded in an rNST-PBN-projecting neuron. A: transient (●) and steady-state (○) outward currents were evoked by a series of depolarization steps to \(-20 \), \(-10 \), and 0 mV following hyperpolarization to \(-110 \) mV to activate \( I_{KA} \). B: steady-state outward current was suppressed by 10 mM TEA; C: transient outward current was blocked by 1 mM 4-aminopyridine (4-AP). TEA- (digital subtraction of B from A) and 4-AP-sensitive (digital subtraction of C from B) components are shown in D and E, respectively. F: voltage protocol.
and Bradley 1994; Uteshev and Smith 2006). Under current-clamp, all I_{KA}-positive neurons responded to a hyperpolarizing-depolarizing current injection protocol (Fig. 4G) with a delay in the initiation of the first spike in the action potential train (41 of 73 I_{KA}-positive neurons; delayed excitation pattern; Fig. 4A) or a prolonged first interspike interval (32 of 73 I_{KA}-positive neurons; ISI spiking pattern; Fig. 4C). Under voltage clamp, these neurons responded to a hyperpolarizing depolarizing voltage protocol (Fig. 4H) with a transient outward current (arrow in Fig. 4, B and D). In contrast, rNST-PBN projection neurons that did not express I_{KA} (n = 28) responded to the hyperpolarizing depolarizing voltage protocol with a train of action potentials to depolarizing current injections (tonic spiking pattern; Fig. 4, E and F).

Expression of I_{KA} by rNST-PBN projecting neurons in CT, IX, and LV terminal fields

There were differences in the distribution of I_{KA}-expressing neurons in the CT, IX, and LV terminal fields. I_{KA}-expressing rNST-PBN neurons predominated in CT (32 of 38 neurons) and IX (27 of 35 neurons) terminal fields. Only ~50% of the rNST-PBN neurons in the LV field expressed I_{KA} (14 of 28 neurons). These differences were significant. I_{KA}-expressing neurons were more frequent in CT (84%) as well as IX (77%) terminal fields than in LV terminal field (50%; \( \chi^2 \) test: \( P < 0.01 \).

Expression of I_h by rNST-PBN projecting neurons

Forty-one percent of all the rNST-PBN projection neurons expressed I_h. I_h-expressing neurons showed a voltage sag (arrow in Fig. 5A) when neurons were hyperpolarized by negative current injection under current clamp. These neurons also had a nonohmic inward current (arrow in Fig. 5B) in response to hyperpolarization under voltage clamp. The nonohmic inward current was eliminated by 3 mM CsCl (n = 6; Fig. 5C), indicating the inward current was I_h (Tell and Bradley 1994; Uteshev and Smith 2006). The amplitude of I_h ranged from 5 to 95 pA as measured by the difference between steady-state and transient currents (○ – ● in Fig. 5B).

Expression of I_h by rNST-PBN projecting neurons in CT, IX, and LV terminal fields

There was no significant difference in the distribution of I_h expression among (n = 19) CT, IX, and LV terminal fields (n = 9; \( \chi^2 \) test: \( P > 0.05 \)). However, the expression pattern of I_{KA} and I_h was significantly different between CT-IX and LV terminal fields. Most I_h-expressing neurons coexpressed I_{KA} in CT (17 of 19 I_h-expressing neurons) and IX (10 of 13 I_h-expressing neurons) terminal fields, whereas only one I_h-expressing neuron coexpressed I_{KA} (1 of 9 I_h-expressing neurons) in LV terminal field (Fisher’s exact test: \( P < 0.01 \)).

Postsynaptic responses of rNST-PBN projection neurons

Postsynaptic currents (EPSC) were recorded from 26 rNST-PBN projection neurons.

ST stimulation above threshold always evoked an EPSC that was of large amplitude and constant waveform with a short mean latency of 3.3 ± 0.2 ms. On repetitive stimulation, the SD of the synaptic latency or “jitter” (Bailey et al. 2006; Doyle and Andresen 2001) was short 155 ± 16 μs (17 of 26 neurons had a jitter <200 μs, and 23 of 26 neurons had jitter values <250 μs). Neurons with a short latency had a corresponding low jitter value (Fig. 6A). The anatomical identification of the neurons in the terminal field and synaptic jitter measurements suggested that the rNST-PBN neurons were monosynaptically connected to the afferent input (Appleyard et al. 2007).

Increasing intensity of current stimuli applied to the ST did not evoke EPSCs with graded amplitudes. Once threshold was exceeded EPSCs had an all-or-none response (n = 15; Fig. 6B).
characteristic of neurons with unitary synaptic input (Araki and De Groat 1996). However, further gradual increase in stimulus intensity recruited additional inputs resulting in a stepwise increase in EPSC amplitude ($n/H_{11005}$; Fig. 6, B and C). Both inputs to this neuron although recruited at different stimulus intensities evoked all-or-none responses.

Morphology of rNST-PBN neurons

Sixty-six PBN-projecting rNST neurons were successfully filled with Lucifer yellow and morphometrically analyzed. Neurons were separated into two morphological groups as either elongate (“E” in Fig. 7) or multipolar (“M” in Fig. 7) based on soma shape, number of primary dendrites and the sites where dendrites originated on the soma (King and Bradley 1994; King and Hill 1993; Lasiter et al. 1989; Mistretta and Labyak 1994; Whitehead 1990). Elongate neurons had fusiform to ovoid cell bodies with two primary dendrites exiting the cell body from opposite poles. Multipolar neurons had cell bodies that were either pyramidal or spherical shaped with three or more primary dendrites distributed in various directions from the soma. These two neurons groups were observed throughout the rNST, but the distribution pattern was significantly different between CT-IX and LV terminal fields. Multipolar neurons were more often encountered in CT (15 of 18 neurons; 83%) and IX (22 of 28 neurons; 79%) terminal fields when compared with the LV terminal fields (9 of 20 neurons; 45%; $\chi^2$ test: $P < 0.05$). Although there were clear differences in neuron biophysics (expression of $I_{KA}$ and $I_h$) as well as morphology (distribution of elongate and multipolar neurons) between CT-IX terminal fields and the LV terminal field, no significant correlation was found between the biophysical and morphological characteristics of the rNST-PBN projection neurons in each terminal field.

DISCUSSION

Sensory information derived from stimulation of taste buds, mechanoreceptors, and thermoreceptors in the oral cavity is relayed to the brain stem and synapses with second order rNST neurons. Sensory processing first begins at this level and recent investigations have revealed how this information is altered and distributed at the brain stem level. Early investigations of rNST neurons assumed that because they responded to stimulation of the oral cavity with chemicals, they functioned as part of the ascending taste pathway involved in taste perception. Moreover, these investigators considered that sensory processing was minimal at the brain stem level consisting of a simple “straight through” connection between first and second order neurons in the taste pathway (Doetsch and Erickson 1970).

More recent studies indicate that considerable sensory processing occurs in rNST. Much new information has been obtained on the biophysical characteristics of rNST neurons, including the properties of afferent synapses and responses of
second-order neurons to synaptic transmitters (reviewed in Bradley 2006; and Smith and Travers 2007). Investigators using anatomical and neurophysiological techniques have established that different populations of rNST neurons project to rostral (Norgren and Leonard 1973) and brain stem locations (Halsell et al. 1996; Travers 1988). Thus sensory information is channeled to CNS areas with very different functions. In addition, evidence derived from selective experimental transection of the afferent input to rNST has demonstrated that facial nerve input is important in sensory discriminatory functions while glossopharyngeal input is related to reflex oral motor rejection circuits (Spector and Travers 2005). Thus connections made by these peripheral cranial nerve branches differ in the way the neural information is distributed.

In the present study we have examined the afferent and efferent connections of rNST neurons projecting rostrally to the PBN. Investigators exploring the characteristics of rNST-PBN projection neurons often use only chemical stimulation of the oral cavity to classify the responses of these neurons, resulting in the conclusion that these neuron only transmit taste information (Cho et al. 2002; Monroe and Di Lorenzo 1995). However, when a broader range of stimuli is used, both chemosensory and somatosensory information is found to be relayed from rNST to the PBN (Norgren and Leonard 1973; Norgren and Pfaffmann 1975; Ogawa et al. 1982, 1984a; Perrotto and Scott 1976). Chemical and mechanical stimuli, applied to the oral cavity evoke responses in ~60% of the rNST-PBN neurons (Ogawa et al. 1984b). A further group of rNST-PBN projection neurons respond exclusively to mechanical stimulation (Ogawa et al. 1984b). In addition, rNST neurons relay thermal information to PBN as well (Ogawa et al. 1968, 1988). Thus in vivo data demonstrate that rNST-PBN projection neurons differ in their responses to orally applied stimuli, perhaps suggesting different groups of neurons participate in parallel information processing. This possibility was suggested in an earlier anatomical study based on the demonstration that two cell types, multipolar and fusiform cells, predominate in the projection from rNST to PBN (Whitehead 1990). Our data also confirm that rNST projection neurons fall into these same two morphological groups. No correlation was found between neuron morphology, intrinsic membrane properties and voltage-gated conductances when attempts were made to group rNST neurons in “blind” patch-clamp recordings (King and Bradley 1994; Uteshev and Smith 2006). Thus rNST neurons with different morphologies can not be separated into groups based on biophysical properties yet can be separated based on responses to chemical, mechanical, and thermal stimulation of the oral cavity. However, the source...
of afferent input and efferent destination of the rNST neurons in these studies was not defined. Our data indicates that rNST-PBN projection neurons in the IX terminal field have a more negative membrane potential and higher input resistance than neurons in the CT and LV terminal fields. The difference in input resistance and membrane potential would affect the voltage-dependent properties of the IX terminal field neurons and possibly relates to the higher proportion of smaller elongate neurons in the IX terminal field.

We have shown that when recordings are made from a defined population of rNST-PBN projecting neurons they can be grouped based on expression of ion channels as well as the type of afferent input they receive. The majority (72%) of rNST-PBN projection neurons express the transient potassium channel—$I_{KA}$. In contrast, when recordings were made from unidentified neurons in rNST, only 37% of the neurons in the medial subdivision that contains the rNST-PBN-projection neurons were $I_{KA}$-positive (Uteshev and Smith 2006). These different results can be explained by differences in the experimental approach. Recordings in the earlier study were made in coronal slices from neurons with unidentified projection patterns.

When the source of input to these neurons is included, 81% of rNST-PBN-projecting neurons located in CT and IX terminal fields (59 of 73 neurons) express $I_{KA}$, whereas only 50% of rNST-PBN neurons in the LV terminal field express $I_{KA}$. Many rNST-PBN projection neurons also coexpress $I_{KA}$ and $I_h$. The majority of rNST-PBN neurons in the CT and IX terminal fields coexpress $I_h$ and $I_{KA}$ while only 4% of the rNST projection neurons in the LV terminal field coexpress $I_h$ and $I_{KA}$. A further group of neurons express $I_h$ alone (5% in the CT, 9% in the IX, and 29% in the LV terminal fields). These results are comparable to the report that very few rNST neurons express only $I_h$ were found more frequently in the lateral subdivision of rNST (Uteshev and Smith 2006).

Functional implications

Most rNST neurons that form the connecting link between the rNST and PBN express $I_{KA}$ and $I_h$, which have been reported to have a marked effect on membrane excitability and action potential firing patterns. $I_{KA}$ influences action potential threshold, action potential duration, and repetitive firing pattern (Storm 2008). Activation of $I_{KA}$ results from a transient change in membrane potential and the GABAergic synaptic input to rNST neurons (Liu et al. 1993; Wang and Bradley 1993) potentially hyperpolarize the neurons to modulate the afferent information being relayed to PBN. Although rNST GABAergic interneurons are the most likely source of the hyperpolarizing inhibitory input, other possible inhibitory inputs include activations of endogenous opiates (Li et al. 2003), dopaminergic neurons (Davis 1998; Davis and Jang 1988; Qian et al. 1997; Zheng and Travaglì 2007), and descending input from rostral brain areas including the cortex (Di Lorenzo and Monroe 1995).

Because $I_{KA}$ inactivates rapidly the early part of gustatory information would be strongly influenced. Many physiological studies have shown that difference of spiking pattern (rate, interspike interval, and synchronicity of spikes) generated by taste stimuli encodes gustatory information in rNST neurons (reviewed in Hallock and Di Lorenzo 2006). Interestingly, the initial action potential discharge pattern in response to taste stimulation contains information sufficient to discriminate among the four basic taste qualities in taste responsive neurons in rat rNST (Di Lorenzo and Victor 2003). rNST-PBN neurons expressing $I_{KA}$ would play an important role in gustatory information processing. $I_h$ also influences repetitive firing and stabilizes the resting membrane potential (Pape 1996). Thus neurons expressing $I_{KA}$ and $I_h$ potentially have an important role in determining firing patterns that code sensory information relayed to PBN via rNST neurons.

Significant differences were found in the expression of $I_{KA}$ in rNST-PBN projection neurons with afferent input derived from oral taste and somatosensory receptors. These differences were also inferred in a study using “blind” patch-clamp recordings based on the known terminations of afferent input to the medial and lateral rNST (Uteshev and Smith 2006). These authors concluded that these differences related to the eventual projection pattern of the rNST neurons. However, in the current study, the recordings are all from rNST neurons that relay to the PBN. Thus it is not possible to conclude that the differences can be explained by projection patterns with diverse functional roles. It is possible that not all sensory input ascending the taste pathway is processed in the same way. Gustatory and somatosensory input is diverse consisting of different taste qualities as well as thermal and mechanosensory stimuli. It is also significant that not all rNST-PBN projecting neurons in the CT and IX nerve terminal fields express $I_{KA}$. This small percentage of neurons would pass on the afferent information relatively unchanged. It is possible therefore that there are a number of parallel sensory pathways between the rNST and the PBN in which the fidelity of the signal is preserved in some neurons and modulated in others.

The synapses responsible for relaying information from the afferent sensory fibers to the rNST-PBN neurons are of the all-or-none type similar to the synapses of the nongustatory NST (Appleyard et al. 2007). This kind of synapse is usually described as a “strong synapse” (Andresen et al. 2004; Franks and Isaacson 2006) because they are characterized by a high fidelity synaptic transmission of information. Thus sensory information reliably crosses the first central synapse in the ascending taste pathway with very little integration of synaptic activity characteristic of graded synapses (Conti and Weinberg 1999). Sensory processing then occurs by local circuit interactions between the second-order rNST neurons and GABAergic interneurons.

In conclusion the rNST-PBN projection relay involves neurons with all-or-none synaptic inputs, heterogeneous neuron morphologies, repetitive discharge characteristics, and expression of ionic conductances. This neuronal complexity reflects the diverse processing functions that occur at the first central relay in the taste pathway.

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