Biophysical and Morphological Properties of Parasympathetic Neurons Controlling the Parotid and von Ebner Salivary Glands in Rats

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Fukami, Hideyuki and Robert M. Bradley. Biophysical and morphological properties of parasympathetic neurons controlling the parotid and von Ebner salivary glands in rats. J Neurophysiol 93: 678–686, 2005; doi:10.1152/jn.00277.2004. The inferior salivatory nucleus (ISN) contains parasympathetic neurons controlling the parotid and von Ebner salivary glands. To characterize the neurophysiological and morphological properties of these neurons, intracellular recordings were made from anatomically identified ISN neurons in rat brain slices. Neurons were also filled with Lucifer yellow and morphometrically analyzed. Based on responses to membrane hyperpolarization followed by depolarization, three types of repetitive discharge patterns were defined for neurons innervating the parotid gland. The regular, repetitive discharge response to membrane depolarization was changed by hyperpolarization resulting either in a delay in the occurrence of the first spike or to an increase in the length of the first interspike interval in the action potential train. Membrane hyperpolarization had little effect on the discharge pattern of some neurons. Similar response discharge patterns were found for neurons innervating the von Ebner salivary gland, which also included a further group of neurons that responded with a short burst of action potentials. Neurons innervating the parotid salivary glands differ morphologically from the von Ebner salivary glands having significantly larger soma and more and longer dendrites than von Ebner gland neurons. In addition, the mean membrane input resistance, time constant, and spike half-width of parotid gland neurons was significantly lower than in von Ebner gland neurons. These differences in intrinsic membrane properties and morphology may relate to the functions of the von Ebner and parotid glands. Von Ebner glands are involved in taste stimulations and removal from posterior tongue papilliae while the parotid glands contribute saliva to the entire mouth.

INTRODUCTION

Parasympathetic secretomotor neurons with efferent fibers that project to the periphery in the glossopharyngeal nerve (IX) are grouped in the inferior salivatory nucleus (ISN). Preganglionic axons originating in the ISN travel in the glossopharyngeal nerve to the otic ganglion from where postganglionic fibers supply the parotid salivary gland (Chibuzo and Cummings 1980; Satomi et al. 1979). Parasympathetic fibers in the glossopharyngeal nerve also travel to the intralingual ganglia (Remak’s ganglia) in the posterior tongue from which postganglionic fibers innervate the von Ebner glands that drain into the clefts of the circumvallate and foliate papillae (Bradley et al. 1980).

The ISN lies principally along the medial edge of the nucleus of the solitary tract (NST) before extending into the ventral reticular formation at its rostral extent (Contreras et al. 1985). This configuration suggests potential interactions between the ISN and NST. However, although the general location of the ISN has been described including brief morphological descriptions of the neurons (Contreras et al. 1980; Satomi et al. 1979), there are no detailed neurophysiological descriptions of ISN neurons and until recently no morphological descriptions (Kim et al. 2004). Moreover, the electrophysiological and morphological characteristics of the ISN neurons have not been related to the glands that they innervate. The purpose of this study was to record from identified neurons in the ISN that innervate the parotid and von Ebner salivary glands, and describe the morphology of the neurons.

METHODS

Retrograde labeling

Twelve-day-old male or female Sprague-Dawley rats were anesthetized with a 6% solution of halothane mixed with air (400–600 ml/min). During surgery, the depth of anesthesia was monitored by the foot withdrawal reflex. All surgical procedures were carried out under National Institutes of Health and University of Michigan Animal Care and Use Committee approved protocols.

The fluorescent retrograde tracer, Alexofluor 568 dextran (Molecular Probes) was used to label ISN neurons innervating either the von Ebner or parotid salivary glands. To label ISN neurons innervating the von Ebner glands, the lingual-tonsillar branch of the glossopharyngeal nerve was exposed by a ventral approach and cut. Crystals of the tracer were applied to the cut central end of the nerve and isolated from surrounding tissue by application of a fast setting silicone sealer (Kwik-sil, World Precision Instruments). To label ISN neurons innervating the parotid gland, the otic ganglion was exposed on the medial aspect of the mandibular division of the trigeminal nerve and crystals of the tracer placed on the ganglion and isolated with silicone. The tissues were then reapposed and the skin sutured. The rats recovered in an isolated cage on a heating pad and when ambulatory were returned to their mother’s home cage.

Brain slice preparation

After a suitable time for retrograde transport (2–4 days), the rats were reanesthetized with halothane and decapitated, and the brain was quickly removed and cooled for 6 min in oxygenated physiological saline in which NaCl was replaced with isosmotic sucrose at 4°C (Aghajanian and Rasmussen 1989). After cooling, the brain was transected at the level of the pons and just below the obex, and the cerebellum was removed. The brain stem was then secured to a Vibratome stage with cyanoacrylate glue and 300-μm-thick horizontal slices cut and transferred to standard physiological saline contain-
ing (in mM) 124 NaCl, 2.5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 26 NaHCO₃, 1.25 KH₂PO₄, and 25 glucose, gassed with 95% O₂-5% CO₂ to give a pH of 7.3. Slices were incubated for ≥1 h in the oxygenated physiological saline at room temperature before being transferred to a recording chamber with a wide-mouthed pipette. Physiological saline at room temperature was flowed at a rate of 1.5 ml/min across the chamber and aspirated at the opposite side. The submerged slice was stabilized by a series of spaced nylon fibers on a wire frame.

Neuropathology

Patch electrodes were pulled from 1.5 mm OD borosilicate filament glass (WPI, TW150F-4) in two stages on an electrode puller (Narashige PP 83). The pipettes were filled with a solution containing (in mM) 130 K gluconate, 10 HEPES, 1 CaCl₂, 1 MgCl₂, and 2 ATP, adjusted to a pH 7.2 with KOH. Lucifer yellow (0.1%, Sigma) was also included in the filling solution for intracellular labeling of the neuron. Filled pipettes had a tip resistance 3–6 MΩ.

Retrodgradely labeled ISN neurons were identified visually under fluorescence illumination using a Nikon E600-FS fixed stage microscope. A ×40 water-immersion objective lens was used to identify and approach a neuron. Once a labeled neuron was identified, it was visualized using infrared-differential interface contrast (IR-DIC) optics via a CCD camera (DAGE IR-1000) (Dodt 1993).

Whole cell recording were performed using an Axoclamp 2B amplifier (Axon Instruments) in bridge mode. When a giga-Ω seal was formed and a patch ruptured, current stimulation protocols were performed and voltage data were acquired using pCLAMP software (Axon Instruments). After recording, the identity of the neuron was confirmed by fluorescence illumination.

Electrophysiological data were analyzed using the Clampfit program (Axon Instruments). Biophysical properties (input resistance, membrane time constants, action potential amplitude, and half-width) were determined by delivering a series of 100-μs hyperpolarizing and depolarizing current pulses (-100 to 75 pA). The junctional potential due to potassium gluconate (10 mM) was subtracted from the membrane potential values (Standen and Stanfield 1992).

We employed an intracellular current injection protocols consisting of membrane hyperpolarization followed by depolarization to separate the ISN neurons into different groups based on their repetitive firing characteristics. A number of other investigators have used this protocol to define different neuron groups based on biophysical characteristics (Bradley and Sweazey 1992; Champagnat et al. 1986; Dekin and Getting 1987; Dekin et al. 1987; Manis 1990; Yarom et al. 1985).

Neuron reconstruction

After completion of recording and cellular labeling, slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and stored for ≥24 h. Slices were then rinsed in this buffer for 30 min, embedded in agar (4% in distilled water) and cut into 100-μm-thick sections on a Vibratome. The sections were mounted on slides, dried overnight, dehydrated by graded alcohols, cleared by xylene and put on coverslips.

Lucifer-yellow-filled neurons were visualized with Bio-Rad MRC- 600 laser scanning confocal microscope. Stacked serial, 1-μm optical confocal images were obtained. Morphometric reconstruction of stacked images was performed using the NeuroLucida program (MicroBrightfield). For each neuron, morphometric measures included soma area, soma form factor (4π area/area), resultings values vary between 0 and 1, where 1 indicates a circular profile and values close to 0 indicate a fusiform shape), total number of primary dendrites, total number of dendritic segments, and total dendritic length. Only one neuron per slice was recorded and 67 neurons were satisfactorily filled to allow characterization and reconstruction.

Data analysis

All analysis was conducted using the SPSS statistical package. Comparisons of different neuron groups were analyzed with one-way ANOVA with Tukey post hoc tests, Student’s paired t-test or χ² test. Correlations were investigated by Pearson’s correlation coefficient and the line fitted by linear regression. Data are presented as means ± SE, and statistical significance was reached at P ≤ 0.05.

RESULTS

The results are based on recordings from ~100 rat pups. Whole cell recordings were made from over 140 neurons in the ISN. Of these neurons 138 were selected because they had a stable resting membrane potential more negative than −40 mV, a spike overshoot of ≥20 mV, and an input resistance >100 MΩ.

Biophysical properties of ISN neurons

| Membrane biophysics of ISN neurons innervating the parotid gland |
|-------------------|-------------------|-------------------|-------------------|
| All Neurons | Delayed Excitation | Long first ISI | Tonic |
| Membrane potential, mV | −58 ± 1.0 | −56 ± 1.0 | −55 ± 1.5 | −45 ± 3.5 |
| Input resistance, mΩ | 373 ± 23.4 | 354 ± 24.8 | 506 ± 57.9 | 355 ± 148 |
| Time constant, ms | 30.1 ± 1.6 | 29.8 ± 1.8 | 32.8 ± 3.9 | 29.6 ± 9.3 |
| Spike amplitude, mV | 95.3 ± 9.5 | 95.7 ± 1.5 | 92.9 ± 2.9 | 94.8 ± 6.3 |
| Spike half width, ms | 1.6 ± 0.7 | 1.5 ± 0.1 | 2.5 ± 0.4 | 1.6 ± 0.05 |
| Number of spikes (100 pA, 100 ms) | 16.2 ± 1.1 | 14.6 ± 1.1 | 24.1 ± 3.1 | 22 ± 6.7 |

n = 55

Values are means ± SE. *Significantly different at the <0.05 level versus delayed excitation (d), long first interspike interval (ISI, l).
occurrence of the first spike (delayed excitation, Fig. 1A) or an increase in the first interspike interval in the action potential train produced by depolarization (long 1st ISI, Fig. 1B). The prehyperpolarization current pulse had the least effect on the discharge pattern of a further group of neurons that responded to the subsequent depolarization by a train of action potential (tonic, Fig. 1C).

Table 1 presents membrane biophysical properties of each group of neurons innervating the parotid gland. Spike half-width and the mean numbers of spikes of delayed excitation neurons were significantly shorter and lower than long 1st ISI neurons (ANOVA: $P < 0.05$). Increasing magnitudes of depolarizing current resulted in an increasing number of spikes for all types of parotid gland ISN neurons (Fig. 2A). Long first ISI neurons responded with a significantly greater number of spikes than delayed excitation neurons.

The duration of the hyperpolarizing prepotential had a marked influence on the number of spikes initiated by depolarization. For delayed excitation neurons, hyperpolarization pulses exceeding 50 ms result in a significant reduction in the mean number of spikes evoked by membrane depolarization (ANOVA: $P < 0.05$). In delayed excitation neurons, spike frequency after the delay was calculated. Hyperpolarizing pulses exceeding 50 ms significantly reduced the mean number of spikes but not spike frequency (Fig. 2B, ANOVA: $P < 0.05$). Membrane hyperpolarization had no significant effect on the mean number of spikes evoked in long 1st ISI and tonic neurons (Fig. 2C).

BIOPHYSICAL PROPERTIES OF ISN NEURON INNERVATING THE VON EBNER SALIVARY GLANDS. Whole cell recordings were made from 83 labeled neurons, and data are summarized in Table 2. Resting membrane potentials ranged from $-40$ to $-73$ mV with a mean of $-56 \pm 0.7$ mV. Input resistance ranged from $275$ to $928 \, \text{M} \Omega$ with a mean of $608 \pm 15 \, \text{M} \Omega$, and membrane time constant was $46 \pm 1.6$ ms. Action potential amplitude ranged from $68$ to $117$ mV with a mean of $93 \pm 1.1$ mV. The mean spike half-width was $2.4 \pm 0.15$ ms.

As shown in Fig. 3, four types of biophysical response patterns have been defined for neurons innervating the von Ebner salivary glands. In addition to the delayed excitation (Fig. 3A), long 1st ISI (Fig. 3B), and tonic patterns (Fig. 3C), a further pattern consisting of a short burst of spikes (burst) not influenced by the hyperpolarization current pulse (Fig. 3D) was found in a few neurons. Table 2 lists membrane biophysical properties of each group of von Ebner salivary gland neuron. Spike amplitude and mean number of spikes of burst neurons are significantly lower than other groups (ANOVA: $P < 0.05$). Spike amplitude of tonic neurons are significantly lower than delayed excitation neurons (ANOVA: $P < 0.05$). Mean number of spikes of delayed excitation neuron was significantly lower than in long 1st ISI and tonic neurons (ANOVA: $P < 0.05$).

Increasing magnitudes of depolarizing current resulted in increasing number of spikes for all types of von Ebner salivary gland neurons except for burst pattern neurons (Fig. 4A). Burst pattern neurons were not affected by increasing magnitudes of depolarizing current. The duration of the hyperpolarizing current injection markedly influenced the number of spikes initiated by depolarization. For delayed excitation and long 1st ISI pattern neurons, membrane hyperpolarization exceeding 50 ms resulted in significant reductions in the mean number of spikes evoked by membrane depolarization (ANOVA: $P < 0.05$). Membrane hyperpolarization had no effect on the mean number of spikes evoked in tonic and burst pattern neurons (Fig. 4B).

FIG. 1. Electrophysiological recordings from inferior salivatory nucleus (ISN) neurons innervating the parotid salivary gland in response to a long depolarizing current injection (a) as well as a short hyperpolarizing pulse followed by a long depolarizing pulse (b). A: an example of a delayed excitation response pattern characterized by a long delay in the action potential train before spikes are initiated. B: an example of a long 1st interspike interval (ISI) response pattern in which there is a long interspike interval between the 1st and 2nd action potential in the action potential train. C: an example of a tonic response pattern in which the prehyperpolarizing pulse has no influence on the pattern of action potentials.
Like neurons innervating the parotid glands, spike frequency of delayed excitation neurons was calculated. Hyperpolarizing pulses exceeding 50 ms significantly reduced the mean number of spikes. Spike frequency after a 150-ms hyperpolarizing current was significantly lower than the frequency resulting from a depolarization not proceeded by a hyperpolarizing current (Fig. 4, ANOVA: P < 0.05). However, spike frequency after a 50- and 100-ms hyperpolarizing pulse did not differ from the frequency resulting from a depolarization not proceeded by a hyperpolarizing current.

**TABLE 2. Membrane biophysics of ISN neurons innervating the von Ebner glands**

<table>
<thead>
<tr>
<th></th>
<th>All Neurons</th>
<th>Delayed Excitation</th>
<th>Long First ISI</th>
<th>Tonic</th>
<th>Burst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane potential, mV</td>
<td>–56 ± 0.7</td>
<td>–56 ± 0.9</td>
<td>–54 ± 1.7</td>
<td>–57 ± 1.5</td>
<td>–52 ± 3.2</td>
</tr>
<tr>
<td>Input resistance, mΩ</td>
<td>608 ± 15.2</td>
<td>589 ± 18.2</td>
<td>674 ± 26.9</td>
<td>607 ± 58.1</td>
<td>609 ± 113</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>46.2 ± 1.6</td>
<td>46.9 ± 1.8</td>
<td>46.6 ± 4.2</td>
<td>45.9 ± 6.5</td>
<td>32.0 ± 2.2</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>92.8 ± 9.9</td>
<td>95.4 ± 1.1</td>
<td>91.3 ± 1.9*</td>
<td>87.1 ± 4.1*</td>
<td>71.7 ± 1.7*</td>
</tr>
<tr>
<td>Spike half width, ms</td>
<td>2.5 ± 0.15</td>
<td>2.5 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.4</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>Number of spikes (100 pA, 1,000 ms)</td>
<td>17.6 ± 0.9</td>
<td>15.9 ± 0.9*</td>
<td>23 ± 1.4*</td>
<td>23.2 ± 2.7*</td>
<td>3.3 ± 1.9*</td>
</tr>
</tbody>
</table>

n = 83 55 16 9 3

*Significantly different at the <0.05 level vs. tonic (t), burst (b), delayed excitation (d), long first ISI (l).

A number of parotid and von Ebner gland neurons exhibit anomalous rectification in response to current injection exceeding –200 pA; 14.5% of parotid gland neurons (8/55) and 56.6% of von Ebner gland neurons (47/83) had anomalous rectifica-

**FIG. 2.** Analysis of the response characteristics of ISN neurons innervating the parotid gland. A: relationship between the number of spikes in response to increasing intensity of current depolarizations for delayed excitation, long 1st ISI, and tonic response pattern neurons. B: effect of the duration of the prehyperpolarization current pulse on the number of spikes generated by a 1,200-ms 100-pA current pulse for delayed excitation, long 1st ISI, and tonic response pattern neurons. C: effect of the duration of the prehyperpolarization current pulse on the mean number of spikes as well as spike frequency for delayed excitation pattern neurons.
tion, and the difference of between the two populations was significant ($\chi^2$ test: $P < 0.05$).

Comparison of the morphological and neurophysiological properties of ISN neurons

Reconstructions were made from 37 parotid and 30 von Ebner gland neurons that were completely filled with Lucifer yellow. Parotid gland neurons had a mean soma area of $207.4 \pm 9.6 \mu m^2$, a form factor of $0.69 \pm 0.08$, and total dendritic length of $660.1 \pm 60.5 \mu m$. On average, they had $4.3 \pm 0.23$ primary dendrites and $9.2 \pm 0.56$ dendritic segments. Von Ebner gland neurons had a mean soma area of $129.1 \pm 7.4 \mu m^2$, a form factor of $0.73 \pm 0.09$, and total dendritic length of $498 \pm 39.1 \mu m$. On average, they had $3.7 \pm 0.15$ primary dendrites and $5.9 \pm 0.3$ dendritic segment. With the exception of the form factor measurement, neurons innervating the parotid salivary glands had significantly larger soma and more and longer dendrites than von Ebner gland neurons (Student’s $t$-test: $P < 0.05$; Fig. 6).

Of the 37 parotid gland neurons, 33 had a delayed excitation pattern (Fig. 7A), 2 neurons had the long 1st ISI pattern (Fig. 7B), and 2 neurons had a tonic response pattern (Fig. 7C). Of the 30 von Ebner gland neurons, 22 had delayed excitation pattern of response (Fig. 7D), 5 neurons had a long 1st ISI pattern (Fig. 7E), and 3 neurons had a tonic pattern (Fig. 7F). There were no correlations between morphometric parameters and response pattern, and there was no significant difference in the distribution of response patterns between parotid and von Ebner neurons.

Correlation between soma area and two membrane biophysical parameters (input resistance and time constant) were analyzed. Both parameters were negatively correlated with soma area (Fig. 8). All the regressions derived are statistically significant ($r = -0.49, P < 0.05$ for input resistance; $r = -0.46, P < 0.05$ for time constant).

DISCUSSION

Neurons of the ISN integrate inputs derived from oral taste and mechanoreceptors as well as from rostral brain areas. The integration results in coded efferent output to control the secretion of the parotid and von Ebner salivary glands. This study is the first report of the electrophysiological and mor-
phological characteristics of identified ISN neurons. ISN neurons clearly differ in morphological and electrophysiological characteristics. Visual inspection of the repetitive discharge patterns from the individual neurons revealed that the ISN neurons could be divided into at least three groups based on the response to a hyperpolarizing followed by a long depolarizing current injection. The first group of neurons (delayed excitation neurons) was characterized by a long delay in their repetitive discharge pattern; the second group (long 1st ISI neurons) was characterized by a long ISI between the first and second spike in the repetitive discharge pattern; the third group (tonic neurons) was characterized by a regular repetitive discharge pattern; and a further small group of neurons responded with a short burst of action potentials at the initiation of the membrane depolarization. The latter group may represent responses recorded from immature neurons as described by other investigators (Bradley and Mistretta 1980; McCormick and Prince 1987) because the recordings were made from relatively young animals.

A surprising finding was the size difference between neurons innervating the parotid and von Ebner salivary glands. ISN neurons innervating the parotid gland were significantly larger than those innervating the von Ebner salivary glands. This size difference is accompanied by differences in membrane properties. The mean input resistance, time constant, and spike half-width of parotid gland neurons was significantly lower than in von Ebner gland neurons. It is possible that these morphological and electrophysiological dissimilarities represent differences in function. Although it is true that activation of the ISN neurons innervating the parotid and von Ebner glands results in secretion of saliva, according to the size principle the smaller cells are more excitable (Clamann and Henneman 1976; Henneman et al. 1965; Luscher et al. 1979). Neurons with smaller cell bodies have a lower threshold for synaptic activation and will therefore respond to weaker synaptic input. Thus ISN neurons innervating the von Ebner salivary glands would potentially be activated with less afferent input than the ISN neurons innervating the parotid gland. Because the von Ebner glands are intimately involved in the delivery and removal of taste stimuli from the clefts of the circumvallate and foliate papillae, rapid reflex coordination between sensory input and secretion would be important in the function of taste receptors situated deeply in the clefts of the papillae. In contrast, parotid glands contribute saliva to the entire mouth and coordination between salivary secretion and afferent stimulation is less critical.

A further difference between neurons controlling the parotid and von Ebner salivary glands is the proportion of neurons that

FIG. 4. Analysis of the response characteristics of ISN neurons innervating the von Ebner salivary glands. A: relationship between the number of spikes in response to increasing intensity of current depolarizations for delayed excitation, long 1st ISI, tonic, and burst response pattern neurons. B: effect of the duration of the prehyperpolarization current pulse on the number of spikes generated by a 1,200-ms 100-pA current pulse for delayed excitation, long 1st ISI, tonic, and burst response pattern neurons. C: effect of the duration of the prehyperpolarization current pulse on the mean number of spikes as well as spike frequency for delayed excitation pattern neurons.
express anomalous rectification. More than 50% of the von Ebner neurons were characterized by having anomalous rectification as compared with less than 15% of neurons innervating the parotid glands. This membrane property results from a cation conductance that is activated by membrane hyperpolarization and serves a number of functions including pacemaker

FIG. 5. Comparison of the electrophysiological properties of ISN neurons innervating the parotid (n = 55) and von Ebner (n = 83) salivary glands. Bars are means and SEs for each parameter. *, the mean of each parameter for parotid and von Ebner neurons are significantly different at the 0.05 level, Student’s t-test.

FIG. 6. Comparison of the morphometric characteristics of ISN neurons innervating the parotid (n = 37) and von Ebner (n = 30) salivary glands. Bars are means and SEs for each parameter. *, the mean of each parameter for parotid and von Ebner neurons are significantly different at the 0.05 level, Student’s t-test.
activity (Pape 1996). Thus it is possible that the resting secretion of saliva that occurs in the absence of any apparent sensory input (Emmelin 1972) could result from oscillatory activity of the ISN neurons that transmit a low level of efferent activity to the glands. A basal level of secretion provided by the von Ebner gland into the clefts of the circumvallate and foliate papillae would be especially important in preventing build up of food debris in the clefts and could account for the larger percentage of neurons innervating these glands that have anomalous rectification. Unfortunately there is no evidence from in vivo studies that these neurons have any oscillatory activity and these neurons are not spontaneously active in the brain slices.

Parasympathetic neurons in the superior salivatory nucleus have repetitive discharge patterns that are similar to those in the ISN (Matsuo and Kang 1998). Most neurons were of the delayed excitation pattern and the rest of the long first ISI pattern. By use of specific blockers under voltage clamp, the current responsible for this pattern was shown to be the fast transient outward potassium current, $I_A$. Most parasympathetic neurons in the dorsal motor nucleus of the vagus nerve also have $I_A$ currents as well, so this appears to be a common characteristic of brain stem parasympathetic neurons (Browning et al. 1999).

The parasympathetic cell column is closely associated with the NST, which is the termination site of the sensory input conveyed in the facial, glossopharyngeal, and vagus nerves. Examination of the repetitive discharge characteristics of both the rostral and caudal NST neurons has shown that neurons of the NST have similar response patterns to neurons of the parasympathetic cell column. NST neurons have been characterized as having delayed excitation, long first ISI, tonic, and burst patterns as well as expressing $I_A$ (Bradley and Sweazey 1992; Dekin and Getting 1987; Dekin et al. 1987; Tell and Bradley 1994). Despite the fact that similar repetitive discharge patterns have been reported in different brain stem areas, the hypothesized functional role of these discharge patterns differs between each of these areas. It is sometimes possible to correlate the patterns defined in vitro to patterns of spike activity recorded extracellularly in vivo. In systems such as the dorsal cochlear nucleus in which in vitro patterns of activity have been documented, the temporal patterns observed in brain slices have similarities to the in vivo patterns (Manis 1990). Unfortunately the response patterns of ISN neurons observed in vitro cannot be compared with patterns recorded in vivo because the only examples of in vivo recordings from ISN neurons result from experiments designed to measure response latency using electrical stimulation of presumed afferent input (Ishizuka and Murakami 1986). Therefore it is difficult to speculate whether these discharge patterns represent patterns that would occur during normal brain stem activity. Moreover, it is also possible by using different temporal patterns of current injection, to demonstrate several repetitive discharge patterns in the same neuron as demonstrated for dorsal cochlear nucleus neurons (Manis 1990).

Efferent output initiated by neurons of the ISN leads to secretion of saliva. Moreover, secretion of saliva is generally elicited only in response to stimulation of the innervation of the glands (Emmelin 1972; Schneyer et al. 1972). Thus afferent input to ISN neurons is required for the initiation and maintenance of secretion. Two types of peripheral stimuli have been shown to be important in initiating secretion. Input originating from stimulation of taste and mechanoreceptors results in the secretion of saliva (Anderson et al. 1985; Kawamura and Yamamoto 1978). In addition the magnitude of the afferent stimulus modulates salivary flow rate (Kawamura and...
Yamamoto 1978). Recordings from the efferent supply to the submandibular gland reveal a linear relationship between impulse frequency and concentration of taste stimuli (Yamamoto and Kawamura 1977).

Details of the brain stem circuits responsible for the reflex secretion of saliva are unclear. Anatomical evidence suggests that there is no monosynaptic input between the afferent fibers and ISN neurons (Whitehead and Frank 1983). Thus afferent fibers first synapse with NST neurons, which then synapse with the ISN neurons directly or via other interneurons in the NST. The afferent input to the NST is excitatory, but inhibitory synaptic activity also occurs within the NST (Bradley and Grabauskas 1998; Li and Smith 1997). Combinations of excitation and inhibition would alter the pattern of response in the ISN neurons as demonstrated by the hyperpolarizing/ddepolarizing current injection protocols. Thus in some circumstances, the output would be of the tonic pattern, whereas in other circumstances, the output would resemble the delayed excitation pattern of discharge. This could account for the different salivary flow rates resulting from sour (acid) stimulation of the tongue when compared with the low flow rates resulting from sweet stimuli (Kawamura and Yamamoto 1978). Because primary afferent taste fibers are grouped into fibers responding best to the different taste modalities (Frank et al. 1983), these fibers conceivably make different synaptic connections to the ISN neurons to control modality specific flow rates.

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References