Effects of 5-hydroxytryptamine and substance P on neurons of the inferior salivatory nucleus

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ABSTRACT

The parasympathetic secretomotor innervation of the salivary glands originates from a longitudinal column of neurons in the medulla called the salivatory nucleus. The neurons innervating the parotid and von Ebner salivary glands are situated in the caudal extremity of the column designated as the inferior salivatory nucleus (ISN). Immunocytochemical investigations have demonstrated the presence of a number of neuropeptides surrounding the ISN neurons. We have examined the neurophysiological effect of two of these neuropeptides on neurons of the ISN identified by retrograde transport of a fluorescent label. Both serotonin (5-HT) and substance P (SP) excited virtually all neurons in the ISN. Application of these neuropeptides resulted in membrane depolarization that was concentration dependent. While the majority of ISN neurons that were depolarized by SP application exhibited an increase in input resistance, application of 5-HT induced widely varied change in input resistance. Membrane depolarization elicited action potential discharges which increased in frequency with increasing concentration of 5-HT and SP. Blocking action potential conduction from surrounding neurons did not eliminate the
depolarizing effects of 5-HT and SP indicating that both neuropeptides acted directly on
the ISN neurons. Finally, the use of 5-HT antagonists and an agonist indicates that 5-HT
acts via a 5-HT$_{2A}$ receptor, and the use of SP agonists suggests that SP acts via
neuokinin-1 and neurokinin-2 receptors. These data show that 5-HT and SP excite most
of the ISN neurons innervating the lingual von Ebner glands possibly modulating the
synaptic drive to these neurons derived from afferent gustatory input.
INTRODUCTION

Saliva secreted into the oral cavity is involved in many functions. It is essential for oral health, mastication and swallowing, speech production and as a solvent and transport medium crucial in the initial steps in taste transduction (Bradley and Beidler, 2003). Saliva also plays a role in protection of the oral mucosa and teeth. In most mammals secretion of saliva is under the reflex control of the autonomic nervous system and is initiated by mastication of food which stimulates oral receptors. Despite the importance of this reflex the neural circuitry responsible for initiation of salivary secretion has received relatively little attention.

The cell bodies of the preganglionic parasympathetic neurons supplying the salivary glands are located in the brainstem salivatory nuclei which are divided into inferior and superior subdivisions based on the cranial nerve distribution of the axons supplying the salivatory glands. Neurons of the inferior salivatory nucleus (ISN) innervate parotid and lingual (von Ebner) glands via the glossopharyngeal nerve while the superior salivatory nucleus (SSN) innervates the submandibular and sublingual glands via the chorda tympani branch of the facial nerve (Loewy, 1990).

Neurons of the salivatory nuclei form the final common pathway of the salivatory reflex (Hector and Linden, 1999). Input derived from stimulating oral taste and mechanoreceptors is one of the major afferent limbs of this reflex system. The sources of other, central input to the salivatory nuclei are located in a large number of rostral brain
areas including the parabrachial complex, Edinger-Westphal nucleus, mesencephalic nucleus, hypothalamus, substantia innominate, bed nucleus of the stria terminalis, and amygdala (Hosoya et al., 1983; Hosoya et al., 1990; Jansen et al., 1992; Takeuchi et al., 1991). Thus, both afferent sensory information as well as descending synaptic input interacts at the salivatory neurons to reflexly initiate salivary secretion (Hector and Linden, 1999; Kawamura and Yamamoto 1978; Matsuo, 1999).

Some details of the brainstem reflex circuitry underlying the salivatory reflex have recently received attention. Neurophysiological and morphological characteristics of the salivatory neurons have been investigated (Fukami and Bradley 2005; Kim et al., 2004; Matsuo and Kang 1998) and more recently investigators have studied the excitatory and inhibitory responses to glutamate and GABA in these neurons (Bradley et al., 2005; Mitoh et al., 2004). However, in addition to glutamate and GABA, these salivatory neurons have been shown to be surrounded by fibers immunostaining for a number of neuropeptides including serotonin (5-HT) and substance P (SP), (Nemoto et al., 1995). In our current work we have shown that neurons of the ISN neurons respond to application of 5-HT and SP.

METHODS

*Retrograde labeling*
Sprague-Dawley rats (10-24 days old) were anesthetized with a 6% solution of halothane mixed with air (400-600 ml/min). Anesthetic level was assessed by lack of a reflex response to mild tail pinching. All surgical procedures were carried out under National Institutes of Health and University of Michigan Animal Care and Use Committee approved protocols.

A fluorescent tracer, Alexa Fluor 568 dextran (Molecular Probes) was used to retrogradely label ISN neurons innervating von Ebner’s lingual salivary glands situated in the posterior tongue, which secrete saliva into the clefts surrounding the circumvallate and foliate papillae. The lingual-tonsillar branch of glossopharyngeal nerve was exposed by ventral approach and cut. Crystals of the fluorescent tracer were applied to the cut central end and isolated from surrounding tissue with silicone sealer (Kwik-Cast, World Precision Instruments). The skin wound was closed with cyanoacrylate glue. Animals recovered in an isolated cage on a heating pad and when ambulatory were returned to the dam's home cage.

*Preparation of brain stem slices*
After 2-4 days the rats were deeply reanesthetized with halothane, decapitated, and the brain rapidly removed and cooled for 6 min in an oxygenated, physiological saline solution in which NaCl was replaced with iso-osmotic sucrose at 4°C (Aghajanian and Rasmussen 1989). The brain stem was transected at the level of the pons and just below the obex, and cemented to a Vibratome (Technical Products International) stage with cyanoacrylate glue and sectioned horizontally into 200 or 300-μm thick slices. The slices were incubated for at least one hour in oxygenated physiological saline solution at 35°C. The physiological saline contained (in mM): 124 NaCl, 5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 26 NaHCO₃, 1.25 NaH₂PO₄, and 25 dextrose; and gassed with a 95% O₂, 5% CO₂ mixture to achieve a solution pH of 7.4.

Recording

A brain slice was transferred to a recording chamber attached to the stage of a Nikon ECLIPSE E600-FN microscope and anchored with a nylon mesh. During recording, the slice was perfused at 2 – 2.5 ml/min with oxygenated physiological saline solution at 35°C. All drugs were dissolved in physiological saline and applied by
perfusion over the brain slices. Drug concentrations were based on those used by others in similar investigations (Browning and Travaglì 1999; King et al., 1993; Lewis and Travaglì 2001).

Retrogradely labeled ISN neurons were identified using brief epifluorescence illumination and then observed using infrared-differential interface contrast optics (IR-DIC) via a CCD camera (IR-1000, DAGE-MTI). A 40x water-immersion objective lens was used to identify and observe the neurons. Whole-cell recordings were obtained from the labeled neurons using a patch-clamp amplifier (Axoclamp-2B, Axon Instruments). Signals were recorded through 2-kHz low-pass filter, digitized at 20 kHz (DigiData 1200, Axon Instruments) and stored on the hard disk of a computer. Data acquisition was performed using pCLAMP 8 (Axon Instruments). Patch pipettes were pulled from borosilicate glass capillaries (TW150F-4, World Precision instruments) using a two-stage puller (PP-83, Narishige) and filled with a pipette solution containing (in mM) 130 potassium gluconate, 10 N-2-hydroxyethylpiperazine – N’- 2 ethanesulfonic 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. Tip resistance of the filled pipettes was 6 – 8 MΩ. The junction potential due to potassium gluconate (10 mV) was subtracted from the membrane potential values (Standen and Stanfield, 1992).

Neurons selected for analysis had a resting membrane potential that was stable and more negative than –40 mV, an action potential overshoot greater than 10 mV and an input resistance greater than 200 MΩ.

Data analysis

All analysis was conducted using the SPSS statistics program. Statistical analysis was performed with a student t-test for comparisons between pairs of groups and by one-way ANOVA with Dunnett post hoc test for comparisons between control and experimental groups. Values in the text and figures are presented as means ± S.E.M. Statistical significance was reached at \( p < 0.05 \). Curves were fitted using the Hill equation.

Drugs
Alexa Fluor 568 dextran was purchased from Molecular Probes (Eugene, OR).

Substance P, 5-hydroxytryptamine, 1- [2-methoxyphenyl /id]-4- [4-(2-phthalimido)-butyl] piperazine (NAN-190), 3- [2-(4- [4-Fluorobenzoyl]-1-piperidinyl]-2, 4 [1H.3H]-quinazolinenedione (ketanserine), 3-tropanyl-indole-3-carboxylate (ICS-205,930), α-
Methyl-5-hydroxytryptamine (α-Me-5-HT), [Sar⁹, Met(O₂)¹¹]-Substance P (SM-SP), α-
Neurokinin (αNK) and tetrodotoxin (TTX) were all purchased from Sigma (St. Louis, MO).

RESULTS

Effects of 5-HT on ISN neurons

The 5-HT results are based on recordings from 106 ISN neurons. Resting membrane potential ranged from –42 to –72 mV with a mean of –55 ± 1 mV. Input resistance ranged from 229 to 965 MΩ with a mean of 497 ± 28 MΩ and action potential amplitude ranged from 71 to 105 mV with a mean of 89 ± 3 mV.

All 106 neurons responded to 5-HT or a 5-HT₂A receptor agonist (α-Me-5-HT) application. Most of the neurons (n = 100; Fig. 1 A and B) responded with a concentration-dependent membrane depolarization while a very few (n = 6) responded by
membrane hyperpolarization. Responses to increasing concentrations of 5-HT were tested in 27 neurons and all were depolarized by 5-HT with a maximum depolarization of 7 ± 2 mV at 30µM and a half maximal response (EC50) at 3.0 µM (Fig. 1C). The depolarization was sufficient to evoke action potentials in 77% of the neurons. Neurons could be separated in two groups based on input resistance changes induced by 5-HT. Some neurons (n = 47) responded with increased input resistance (EC50 = 2.7 µM, Fig. 1D) while others (n = 53) responded with a decrease (EC50 = 2.6 µM, Fig. 1E) even though all were depolarized by 5-HT application.

Application of either 5-HT (50 µM in 3 neurons and 100 µM in 1 neuron) or α-Me-5-HT (50 µM in 2 neurons) resulted in membrane hyperpolarization. The hyperpolarization by 5-HT averaged 3 ± 1 mV accompanied by a decrease in both input resistance and number of action potentials elicited by a depolarizing current injection (60 pA, 100 ms, data not shown).

Action potentials were evoked by a long depolarizing current injection (50 pA, 1200 ms duration). To eliminate the influence of depolarization induced by 5-HT on
voltage-gated channels, neurons were maintained at -50 mV by current injection.

Compared to current injections in control buffer, application of 100 µM 5-HT increased the number of evoked spikes from 11 ± 1 spikes to 17 ± 1 spikes (n = 8; t-test: p < 0.05) independent of any change in input resistance (an increase in 2 and a decrease in 6 neurons). In addition, the increased spike frequency was accompanied by a decrease in the time to initiate the first spike from 21 ± 3 ms to 17 ± 2 ms (n = 7; t-test: p < 0.05) and a decrease in the interval between the first and second spike from 66 ± 4 to 47 ± 5 ms (n = 9; t-test: p < 0.05) (Fig. 2). 5-HT therefore increased the excitability of the ISN neurons.

To investigate whether 5-HT depolarizes the ISN neurons directly or via other neurons synaptically connected to the neurons, we applied 5-HT (100 µM) in the presence of tetrodotoxin (TTX, 2 µM) to block conduction of action potentials. In five neurons tested, the magnitude of the depolarization after 5-HT application was 5 ± 1 mV which is similar to the results of 5-HT application in control saline indicating that the
depolarizing effect of 5-HT was independent of action potential mediated synaptic
transmission.

5-HT receptors are composed of a number of subtypes (Barnett and Sharp 1999;
Hoyer et al., 2002) and we used several 5-HT receptor subtype antagonists to determine
which subtypes are involved in the effects of 5-HT on the ISN neurons. Based on
experiments on preganglionic parasympathetic neurons of the dorsal motor nucleus of
vagus (Browning and Travagli 1999) which is a caudal extension of the ISN, we
investigated the involvement of 5-HT$_{1A}$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors. 5-HT (50
µM) was applied in the presence of one of these antagonists. As shown in Fig. 3, a 5-
HT$_{2A}$ receptor antagonist, ketanserin (10 µM) blocked or largely suppressed the
depolarizing effect of 5-HT ($n = 13$; ANOVA: $p < 0.05$). A 5-HT$_{1A}$ receptor antagonist,
NAN-190 (10 µM; $n = 8$) and a 5-HT$_{3/4}$ receptor antagonist, ICS-205,930 (10 µM; $n = 9$)
had no significant effect on membrane depolarization by 5-HT. To confirm involvement
of 5-HT$_{2A}$ receptors in the 5-HT response, a specific 5-HT$_2$ receptor agonist, α-Me-5-HT
(50 µM) was applied which mimicked the depolarizing effect of 5-HT ($n = 6$). Thus, 5-
HT acting via 5-HT$_{2A}$ receptors depolarizes the ISN neurons. In two additional neurons, application of α-Me-5-HT resulted in membrane hyperpolarization with a decreased input resistance similar to the group of four neurons already described that responded to 5-HT by membrane hyperpolarization. This result suggests that hyperpolarization induced by 5-HT may be mediated by 5-HT$_2$ receptors. Because we encountered so few ISN neurons that were hyperpolarized by 5-HT we were not able to investigate this effect further.

Effects of Substance P on ISN neurons

The results of the responses to SP were derived from 32 ISN neurons. All of the neurons tested responded to SP by membrane depolarization and the magnitude of the depolarization increased with concentration (Fig. 4). The maximum depolarization and input resistance increase induced by 1 μM SP was $9 \pm 1$ mV and $33 \pm 10$ MΩ ($n = 10$) respectively. The half maximal response occurred at 0.01 μM SP (Fig. 4C). Depolarization was accompanied by an increase in input resistance in 27 of the neurons ($42 \pm 7$ MΩ at 1 μM). In the remaining neurons ($n = 5$), input resistance did not apparently change. SP at concentrations of 1 μM and higher resulted in the generation of
spontaneous action potentials in most of the neurons (19/21) (Fig. 4A). To determine if SP acts directly on the ISN neurons, 1 µM SP was applied in the presence of 2 µM TTX. In three neurons tested, the magnitude of depolarization induced by SP was 8 ± 1 mV in control and 6 ± 1 mV in the presence of TTX, and the increase in input resistance was 120 ± 41 MΩ in control and 77 ± 9 MΩ in the presence of TTX (data not shown). These results indicate that SP has direct postsynaptic action on the ISN neurons.

Application of SP increased the action potential discharge rate (Fig. 5). Action potentials were evoked by depolarizing current injection (100 pA, 800 ms duration). To eliminate effect of depolarization induced by SP on voltage-gated channels, neurons were maintained at resting membrane potential by current injection. This experiment was performed in the neurons that did not show an obvious increase in spontaneous synaptic activity by application of SP. In 14 neurons, the number of action potentials resulting from the depolarizing current injection was significantly increased from 10 ± 1 in control saline to 13 ± 1 after SP application (t-test: p < 0.05). Also, the time to initiate the first action potential and the first interspike interval were significantly decreased from 15 ± 2
ms to 13 ± 1 ms and from 48 ± 3 ms to 44 ± 2 ms in control and SP containing saline respectively (t-test: \( p < 0.05 \)). These results indicate that SP changes membrane conductance and increases the excitability of the ISN neurons.

To investigate receptors which mediate the effect of SP, a neurokinin-1 receptor agonist SM-SP and a neurokinin-2 receptor agonist \( \alpha NK \) were used. In 6 neurons, 1 \( \mu M \) SP induced 8 ± 1 mV depolarization and a 13% increase in input resistance. After wash out of SP, application of 3 \( \mu M \) SM-SP induced a similar depolarization of 7 ± 1 mV and an 11% increase of input resistance (Fig. 6A and Ba). In a further group of 6 neurons application of 3 \( \mu M \) \( \alpha NK \) induced a 9 ± 3 mV depolarization and an 8% increase of input resistance (Fig. 6A and Bb). There was no significance difference between the value of the membrane potential change due to SP and the agonists applications (ANOVA: \( p > 0.05 \)) and there was no significance difference in the percentage-change of input resistance between application of SP, SM-SP and \( \alpha NK \) (t-test: \( p > 0.05 \)). These results indicate that both neurokinin-1 and 2 receptors are involved in the effect of SP on ISN neurons.
DISCUSSION

We have determined that neurons of the ISN respond to both 5-HT and SP. Our data indicates that the excitatory effects of 5-HT and SP are probably due to direct postsynaptic action on the ISN neurons because the response persists in the presence of TTX to block of action potential conduction and therefore indirect excitation from adjacent neurons. The excitation by 5-HT was mediated by activation of the 5-HT$_{2A}$ receptor while SP excitation was mediated via both neurokinin-1 and 2 receptors. The effects of 5-HT and SP were dose-dependent and are similar to the results of investigations of the preganglionic parasympathetic neurons of the dorsal motor nucleus of the vagus (Browning and Travagli 1999; Lewis and Travagli 2001) which extends caudally from the salivatory nuclei.

While 5-HT depolarized all ISN neurons, membrane conductance changes were divergent. Parasympathetic neurons of the salivatory and dorsal motor nucleus of the vagus are of heterogeneous morphology (Fox and Powley 1992; Kim et al., 2004) and biophysical properties (Yarom et al., 1985; Tell and Bradley 1994; Travagli and Gillis
1994; Matsuo and Kang 1998; Fukami and Bradley 2005), some neurons having predominant $I_{A}$, while others have $I_{KIR}$ currents. Other ion channels have also been identified in these parasympathetic neurons involved in the excitatory effect of 5-HT (Hopwood and Trapp 2005). Depolarization of these neurons by 5-HT would potentially activate these and other voltage-dependent channels resulting in the divergent conductance changes. Functionally, different populations of ISN neurons would potentially modulate excitatory and inhibitory input in different ways.

The source of the immunostained neuropeptides surrounding the ISN neurons is assumed to be from either brainstem or more rostral brain areas (Nemoto et al., 1995). However, both 5-HT and SP immunoreactive neurons are described in the petrosal ganglion which contains the cell bodies of the gustatory input from the posterior tongue (Helke and Hill 1988; Okada and Miura 1992; Zhong et al., 1999). While some of these petrosal ganglion neurons may be associated with the afferent innervation of the carotid body not all the SP positive neurons are carotid body neurons (Finley et al., 1992). It is conceivable, therefore, that some of the immunohistochemical 5-HT or SP staining
associated with the salivatory neurons may originate from the afferent input to the NST as has been suggested for neurons involved in the baroreceptor reflex (Raul 2003). The NST also immunostains for 5-HT receptors (Thor et al., 1988; Manaker and Verderame 1990; Thor et al., 1992) and neurons of the rNST respond to SP (King et al., 1993). Thus, superfusion of the brain slice with 5-HT and SP would stimulate neurons in the NST which synapse with the ISN neurons and this may be reflected in the increased synaptic activity evoked by SP in Fig. 4Bb.

Action of 5-HT and SP on other brainstem parasympathetic reflexes

The role of 5-HT and SP in the baroreceptor reflex arc has been extensively studied (Jordan 2004; Raul 2003). Microinjection of 5-HT into the caudal NST elicits the typical responses of baroreceptor activation, mediated by activation of 5-HT$_2$ postsynaptic receptors. As in the present study 5-HT application results in both excitatory and inhibitory effects on baroreceptor reflex neurons (Sevoz-Couche et al., 2000); the excitatory effect is mediated by 5-HT$_{2A}$ receptors and 5-HT$_{2C}$ receptors are responsible for inhibition. We were not able to record from sufficient inhibitory (hyperpolarizing)
ISN neurons to determine which 5-HT receptor was expressed in these neurons but all
excitatory (depolarizing) neurons expressed 5-HT$_{2A}$ receptors. Thus, there are similarities
between the neurobiology of the baroreceptor and gustatory-salivatory reflex arcs. Both
involve afferent input to the NST and efferent parasympathetic motor output neurons. It is
possible then to conclude that as in the baroreceptor reflex, the role of 5-HT in the
gustatory-salivatory reflex is as a facilitator.

SP also excites other brainstem nuclei including neurons of the caudal and rostral
nucleus of the solitary tract (Champagnat et al., 1986; King et al., 1993) which receive
input from sensory afferents as the major input component of reflex activity of the oral
cavity and gut. The output cells of this reflex are also sensitive to SP. According to Lewis
and Travagli (Lewis and Travagli 2001) the SP containing pathways from both the
peripheral and central nervous system are involved in the vagal neurons controlling
gastric motility.

*Role of the ISN neurons in control of the von Ebner glands*
The basic function of the ISN neurons examined in the current study is to integrate information from oral receptors and a number of brain areas to control the secretion of the von Ebner salivary glands. In contrast to major salivary glands which secrete directly into the oral cavity von Ebner glands secretions empty into the clefts of the posterior tongue gustatory papillae. Since the epithelium of the clefts contains hundreds of taste receptors (Miller 1977; Miller and Smith 1984) it has always been assumed that these glands have an important role in taste function. Due to the position of the taste receptors in the cleft epithelium, taste stimuli have to somehow access the taste receptors from the oral cavity. Secretions of the von Ebner’s glands provide a diffusion path facilitating the transport of taste stimuli. In addition the von Ebner secretions flowing from the gland ducts into the clefts also function to flush out the clefts to remove taste stimuli as well as food debris thus maintain a healthy environment. These potential roles of the secretions of von Ebner’s glands in taste function have been tested experimentally (Gurkan and Bradley 1988). Stimulation of von Ebner gland secretion
reduced taste responses evoked from the posterior tongue taste buds presumably via a mono or polysynaptic reflex connecting the afferent sensory input to the ISN neurons.

The afferent limb of this reflex involves glutamate receptors (Bradley et al., 2005). However, reflex secretion of saliva also results from other sensory inputs such as olfaction (Hector and Linden, 1999), vision and “psychic” factors are also thought to act as initiators of salivary secretion (Holland and Matthews 1970). Thus, there are many potential pathways involved in salivary secretion and these may use different neurotransmitters and neuromodulators. It is therefore reasonable to suggest that 5-HT and SP have a role in either maintaining the resting flow rate or synaptic mediators of one of the descending pathways originating from other sensory systems.
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References


Figure legends

**FIG. 1.** Membrane responses to application of 100 μM 5-HT recorded in an ISN neuron.

**A:** Upper trace shows responses to constant -100 pA 100 ms 0.5 Hz hyperpolarizing current pulses (lower trace) during application of 5-HT. Arrowhead above the upper trace shows onset time of a 1 min 5-HT application. Numbers above the upper trace are the membrane potential values before and after application of 5-HT. Hyperpolarizing (-100 pA, 800 ms) and depolarizing (20 pA to 100 pA in 20 pA increments, 800 ms) current pulses were injected before (a) and after (b) application of 5-HT to measure membrane resistance. **B:** Faster time base recordings from Fig. 1A. Responses to the hyperpolarizing (-100 pA) and depolarizing (40 pA) pulses before (a) and after (b) application of 5-HT (spikes truncated). 5-HT dose-response relationships between 5-HT concentration and membrane potential (**C**) and input resistance (**D** and **E**). **D** shows an increase and **E** shows a decrease in input resistance induced by increasing concentrations of 5-HT respectively. Numbers of measurements are shown above each data point.
FIG. 2. Influence of 5-HT on action potential frequency. A: Action potentials evoked by injection of depolarizing currents (50 pA, 1200 ms) in control (upper trace) and during application of 100 µM 5-HT (lower trace). B: Action potentials from A at a faster trace speed. The neurons were maintained at -50 mV by current injection before the depolarizing current injections.

FIG. 3. Effect of 5-HT receptor antagonists and an agonist on ISN neuron membrane potential. Bars show values of membrane potential change by application of 5-HT in control, in the presence of 5-HT receptor antagonists and by application of α-Me-5-HT. Numbers of neurons tested are shown above each bar.

FIG. 4. Membrane responses to 0.1 µM SP recorded from an ISN neuron. A: Upper trace shows responses to constant -100 pA, 100 ms, 0.5 Hz hyperpolarizing current pulses (lower trace) during application of SP. An arrowhead above the upper trace indicates onset time of a 1 min SP application. Numbers above the upper trace are the membrane potential values before and after application of SP. Hyperpolarizing (-100 pA, 800 ms) and depolarizing (20 pA to 100 pA in 20 pA increments, 800 ms) current pulses were...
injected before and after application of SP to measure membrane resistance (a and b). B:

Faster time base recordings from Fig. 4A. Responses to hyperpolarizing (-100 pA,) and depolarizing (60 pA) current injections before (a) and during (b) application of SP. C:

Relationship between SP concentration and membrane potential. Values of each membrane potential change were normalized with respect to maximal response and plotted against concentration of SP. Numbers of measurements are shown above each data point. Note that some neurons (8 of 32 neurons tested) showed an increase of spontaneous synaptic activity after SP application (Bb).

Fig. 5. Influence of SP on evoked action potential frequency. Membrane response evoked by hyperpolarizing current (-100 pA, 800 ms) and depolarizing current (100 pA, 800 ms) respectively (lower traces) before (A) and after (B) 1 min application of 1µM SP. In Fig. 5A the depolarization due to SP was returned to the resting membrane potential value of -52 mV by current injection.

Fig. 6. Membrane responses to application of neurokinin receptor agonists SM-SP (3µM) and αNK (3µM). A: Depolarization resulting from application of the agonists. The
values were normalized with respect to the amplitude of depolarization resulting from 1 µM SP application. *Ba and Bb:* Comparisons of the input resistance change induced by SP and the two neurokinin agonists.