Sensory Afferent Neurotransmission in Caudal Nucleus Tractus Solitarius—Common Denominators

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Abstract

The nucleus of the solitary tract (NTS) receives a wide range of sensory inputs including gustatory, gastrointestinal and cardiorespiratory which are loosely segregated viscerotopically to subnuclei. Our laboratory has focused on a dorsomedial area of caudal NTS (mNTS) which is critical for cardiovascular reflexes. Using a brainslice, we study primarily mNTS neurons mono-synaptically activated by solitary tract stimulation. mNTS neurons show varying degrees of delayed excitation, spike frequency adaptation and after hyperpolarizations. Sensory afferent transmission is mediated by glutamate acting at post-synaptic non-NMDA receptors. Glutamate release depends on at least four different presynaptic calcium channels with N-type predominating. This profile of presynaptic calcium channels in NTS is also present at the peripheral soma, but absent from the baroreceptor sensory endings. Many peptides are associated with these sensory neurons and several modulate glutamatergic transmission in mNTS. Angiotensin II facilitates excitatory responses to sensory afferent activation by a presynaptic mechanism. Caudal NTS appears to have a framework of synaptic and cellular mechanisms in common with other NTS areas and peptides may play a critical role modulating this framework. **Chem. Senses 21: 387–395, 1996.**

The nucleus of the solitary tract (NTS) is a major site of initial integration of a wide range of sensory neuron inputs including gustatory, gastrointestinal, cardiovascular and respiratory. This single anatomical nucleus has a number of distinct cell types distributed across and within subnuclei within the borders of NTS. The various sensory inputs to NTS are loosely segregated viscerotopically to subnuclei along the rostral-caudal extent of NTS (Loewy, 1990). Overlap of these various regional sensory inputs appears to increase caudally within NTS which may indicate increased sensory convergence. Rostral regions of NTS receive sensory information from the tongue, epiglottis and soft palate. In intermediate NTS, adjacent to the area postrema and near the midline, parvicellular NTS receives gastrointestinal sensory inputs and cardiovascular afferents synapse primarily in

medial NTS adjacent to the tract, whereas respiratory tract and pulmonary sensory afferents synapse in lateral and ventrolateral aspects of intermediate NTS. In the caudalmost region of NTS, cardiovascular, gastrointestinal, respiratory tract and pulmonary afferents synapse in a broad mediolateral pattern. With such rich innervation, NTS is the site of the first central synapses and integrative center for a number of vitally important homeostatic reflexes (Seller and Illert, 1969; Onai *et al.*, 1987; Andresen and Kunze, 1994).

Our laboratory is interested in cardiovascular regulation and has focused on a dorsomedial area of caudal NTS (mNTS) which is critical for cardiovascular reflexes and receives a dense innervation from aortic arch baroreceptors (Mendelowitz *et al.*, 1992). The presence of arterial baroreceptor synapses in this region has been indicated in a

range of species by anatomical and electrophysiological means (Andresen and Kunze, 1994). Since intracellular recordings from these small neurons *in vivo* is relatively difficult, we developed an *in vitro* brain slice preparation of rat mNTS in which we could selectively activate sensory axons in the solitary tract (ST) and record from mNTS neurons. By orientating our slice in a roughly horizontal fashion, we preserved relatively lengthy sections (1–3 mm) of ST in the same plane as the mNTS neurons. By stimulating relatively distant from the recorded cell bodies, electrical activation of ST axons was selective and free of recruitment of local interneurons (Andresen and Yang, 1995a). Our general experimental strategy was to initially classify neurons functionally by their response to ST stimulation. Generally, we found three responses characteristically evoked by ST activation: short-latency (<3 ms) excitatory post-synaptic potentials (EPSPs), long latency (>5 ms) inhibitory PSPs (IPSPs) and more complex, EPSP-IPSP sequences (Andresen and Yang, 1995a).

Most of our studies to date have focused primarily on mNTS neurons mono-synaptically activated with an EPSP by ST stimulation. Such responses are believed to reflect the activation of primary sensory afferent neurons at the first central synapse. One of the most basic issues concerning

neuronal function in NTS is the presence and relative expression of various ion channels, and how these give rise to the basic discharge properties of these mNTS neurons. Several studies have examined general discharge properties of NTS neurons and many similar patterns are reported throughout the rostral-caudal extent of NTS (Dekin and Getting, 1984, 1987; Champagnat *et al.*, 1986; Dekin *et al.*, 1987; Haddad and Getting, 1989; Bradley and Sweazey, 1992; Richter *et al.*, 1993; Champagnat and Richter, 1994; Tell and Bradley, 1994). As a result this suggests fairly similar ensembles of membrane ion channels, particularly the various potassium selective varieties. Delayed excitation (DE) is broadly observed across the rostral-caudal extent of NTS. In mNTS neurons receiving short latency EPSPs to ST stimulation (Schild *et al.*, 1993), depolarizing current injection evokes an increased discharge which rapidly adapts (<300 ms) to a new, elevated steady state discharge level (Figure 1, right most traces) in a process termed spike frequency adaptation (SFA). Injection of hyperpolarizing current in these same neurons briefly interrupts ongoing discharge, but does not materially alter the prevailing rate (Figure 1, left most traces). However, preceding the injection of depolarizing current by a conditioning hyperpolarizing pulse (Figure 1, middle traces) delays the onset of discharge
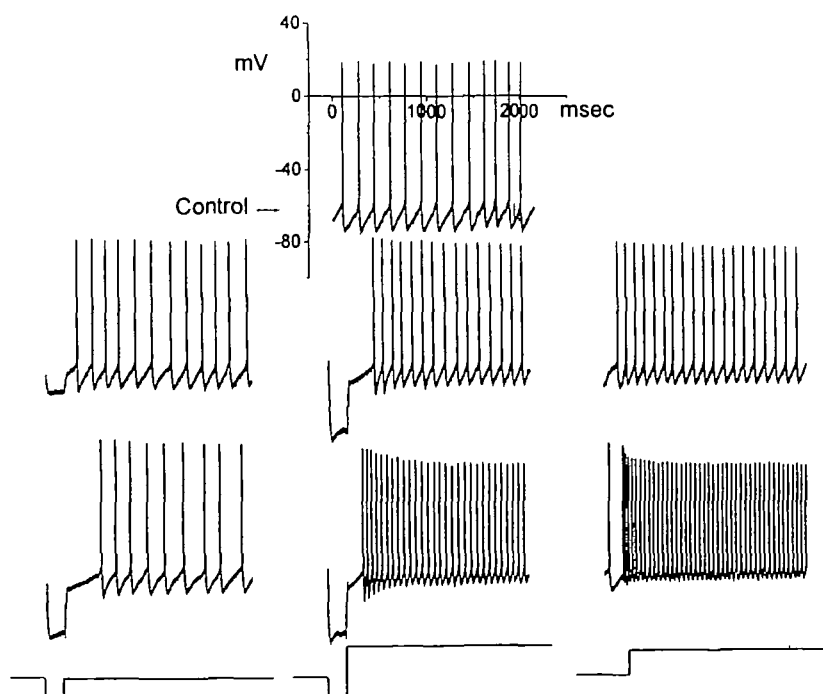
Figure 1 Presence of delayed excitation typical of mNTS neurons receiving short-latency, EPSPs from ST activation. Upper trace shows the resting, undisturbed discharge pattern of this neuron. The lower traces show from left to right, responses: (i) to hyperpolarizing current injection, (ii) to hyperpolarizing current injection followed by depolarizing current injection and (iii) to depolarizing current injection alone—as indicated by the lower line tracings. All traces scaled identically as in upper control trace. Resting potential was approximately −60 mV.

to the depolarizing current (i.e. DE), reduces the discharge rate attained during the depolarizing current and can greatly reduce or eliminate SFA. Various neurons in mNTS with excitatory connections to ST all show DE and SFA, but to varying degrees. Using a comprehensive mathematical model representation of mNTS neurons, we have coupled data from isolated currents measured in isolated, dispersed mNTS neurons with responses recorded from neurons in mNTS slices. Such studies show that DE and SFA result from a critical interaction of two transient outward, A-type potassium currents withdiffering kinetics (Schild *et al.*, 1993), and gives rise to this voltage and time dependent behavior.

Thus, the post-synaptic NTS neurons appear to have a conventional complement of voltage dependent ion channels (Champagnat *et al.*, 1986; Schild *et al.*, 1993). TTX eliminates action potentials in most NTS neurons indicating the presence of a fast sodium current I_{Na} (Andresen and Kunze, 1994). Two calcium currents can be resolved, an L-type and a T-type (Kunze, 1987). Several potassium channels have been identified in NTS. A slowly developing, non-inactivating potassium current, I_K, is blocked by TEA (Dekin and Getting, 1987; Moak and Kunze, 1993). A TEA-resistant, transient potassium current, I_A, and a delayed, more slowly inactivating potassium current, I_D, are more variably present across NTS and are blocked by 4-AP (Moak and Kunze, 1993; Andresen and Kunze, 1994). An $I_{K,Ca}$ is present in mNTS neurons (Moak and Kunze, 1993; Andresen and Kunze, 1994) and is blocked by charybdotoxin (Moak and Kunze, 1993).

These three major classes of intrinsic time- and voltage-dependent ionic channels, sodium, calcium and potassium, give rise to a spectrum of response properties in NTS neurons, and are capable of transforming the output of NTS neurons in response to synaptic activation or inhibition (Schild *et al.*, 1993). No absolute differential distribution of these channels across the subnuclei of NTS is apparent, although subtypes of NTS neurons have been suggested based on morphology and/or on discharge characteristics such as SFA and DE which depend on these channels (Andresen and Kunze, 1994). Short periods of conditioning at hyperpolarized membrane potentials greatly dampens the response to subsequent activation for prolonged intervals and produces the signature DE response prominently featured in most portions of NTS (Yang and Andresen, 1992; Schild *et al.*, 1993; Andresen and Kunze, 1994). Potassium channel blockers support a prominent general role of I_A in DE (Dekin and Getting, 1984, 1987). In addition, these or other voltage-gated channels are targets for selective modulation by neurotransmitters (Bacal and Kunze, 1991; Priddy *et al.*, 1992).

Micro-injection, immunocytochemical and electrophysiological evidence implicates a variety of potential transmitters and modulators in caudal medial NTS (Van Giersbergen *et al.*, 1992; Andresen and Kunze, 1994). Major classes are:
(i)   biogenic amines;
(ii) amino acids;
(iii) neuropeptides.
Evidence for most potential transmitters is largely based on anatomical localization or indicated by changes in blood pressure (BP) and heart rate on injection into NTS (Van Giersbergen *et al.*, 1992). The two most prominent transmitters are glutamate (Glu) and γ-aminobutyric acid (GABA). As in other CNS areas, Glu mediates fast excitatory synaptic transmission (Talman *et al.*, 1980), and GABA is associated with pre- and post-synaptic inhibition. Sensory afferent soma contain many other transmitter related substances (for reviews see Helke and Hill, 1988; Helke and Neiderer, 1990).

Excitatory amino acids activate two basic types of response systems: ion channels (ionotropic) and second messenger systems (metabotropic) (Nicholls, 1992). Based on agonist preferences, three classes of inotropic receptors are distinguished: N-methyl-D-aspartate (NMDA) and two non-NMDA classes, AMPA and kainate. Baroreflexes are blocked by micro-injection of broad-spectrum Glu antagonists into NTS (Andresen and Kunze, 1994; Ohta and Talman, 1994). Micro-injection of Glu or its analogs into NTS evokes BP responses and responses to AND stimulation are blocked by selective NMDA antagonists (Andresen and Kunze, 1994). Recent reflex work, however, concludes that non-NMDA receptors predominate in NTS (Andresen and Kunze, 1994; Ohta and Talman, 1994). In our NTS slice, short-latency EPSPs to ST stimulation are blocked by non-NMDA antagonists (Figure 2), but are unaffected by NMDA receptor or channel antagonists (Andresen and Yang, 1990, 1994). Thus, it appears likely that for Baroreceptor, as well as other sensory afferents, fast synaptic transmission is mediated primarily by Glu acting at a non-NMDA receptor.

Post-synaptic NMDA responses are apparent, however, within intermediate and caudal NTS. Small NMDA currents (<15 pA) are evoked in most dissociated medial NTS neurons (Andresen and Kunze, 1994). Only a minority (18%) of cells, however, respond to NMDA with substantial currents. No contribution of NMDA to ST-NTS EPSPs is present in horizontal slices. In transverse slices, stimulation of sites off and medial to the ST elicit a small, slow EPSP
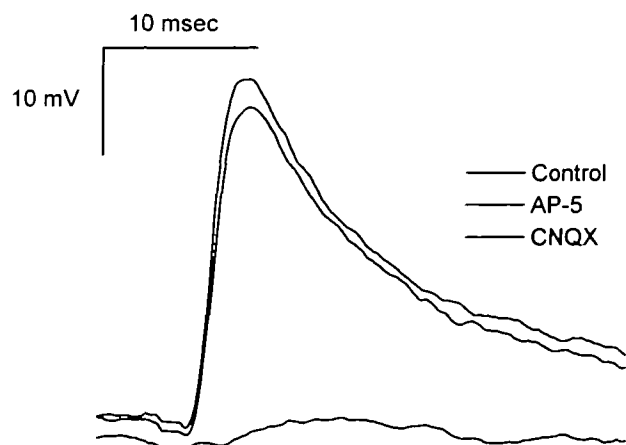
Figure 2 Short-latency, EPSP from ST activation under control (uppermost trace) conditions or in the presence of AP5 (100 μM), a specific NMDA antagonist or CNQX (10 μM), a specific non-NMDA antagonist. The EPSP is mediated almost entirely by non-NMDA receptors with little evidence of any residual response

in NTS neurons which is blocked by NMDA antagonist (Brooks and Spyer, 1993). These results suggest that NMDA receptors may be on interneurons within NTS, but are relatively absent from cells receiving primary afferent inputs. Such an hypothesis would give a cellular explanation for the complexity of BP responses to NMDA micro-injected into NTS.

After block of NMDA and non-NMDA receptors, small BP responses to injection of Glu into NTS remain (Andresen and Kunze, 1994). Recent *in vitro* experiments indicate that metabotropic Glu receptors are present in NTS (Glaum and Miller, 1992). After NMDA/non-NMDA block, trans-ACPD (metabotropic agonist), Glu and ST stimulation elicit small depolarizations and modulation of GABA and AMPA responses in NTS neurons in transverse slices (Glaum and Miller, 1992, 1993a). The metabotropic antagonist MCPG turned frequency dependent depression into facilitation (Glaum and Miller, 1993b, 1994). Interestingly, and in contrast, in our horizontal slices, MCPG depresses low frequency ST-NTS EPSPs and does not affect the frequency response relation; the opposite of the apparent results in transverse slices (Andresen and Yang, 1995b).

Transmitters may have as targets presynaptic sites on the sensory afferent terminals within mNTS. These presynaptic receptors may be ion channels opened by e.g. Glu, GABA or glycine receptors. Alternatively, ligand-activated receptors may, directly or through second messenger systems, modulate the activity of voltage-dependent channels in the membrane of the central presynaptic terminal. In either case, the

final common pathway for presynaptic modulation is via regulation of intracellular calcium and subsequent transmitter release. A key question to understanding the presynaptic modulation of synaptic transmission between the sensory fiber and the NTS neuron is: what ion channels control transmitter release? The central terminals are not currently accessible to electrical recording. However, presynaptic mechanisms can be inferred indirectly from post-synaptic responses measured in NTS neurons. An alternative and, in some ways, more direct approach is to identify and characterize the channels at the cell body of these sensory neurons (Llinás, 1988). Channels expressed in the soma may well be present at the central synaptic terminal. Information about these channels can then used to probe the synaptic terminals with techniques such as localizing labeled high affinity agonists for a particular channel to the synaptic terminal region or by monitoring the EPSP in the presence of agents that block specific ion channels. Sodium and particularly potassium channels are also of great interest and have been studied in nodose and baroreceptor neurons (Andresen and Kunze, 1994).

Calcium currents are of special interest as they are often the target of modulation by neurotransmitters. At least three types of calcium channels are present in the baroreceptor and other sensory neuron soma. A small component of the total calcium current is contributed by a low threshold T-type channel Ca_T (Andresen and Kunze, 1994). High threshold calcium current is present in unidentified sensory soma (Andresen and Kunze, 1994). In nodose ganglia, an ω-conotoxin GVIA (CTX)-sensitive channel (Mendelowitz and Kunze, 1992) and a quiescent pool of dihydropyridine (DHP) sensitive channels (Bacal and Kunze, 1994) are both present. A ω-conotoxin GVIA-sensitive calcium current is modulated by Angiotensin II (Bacal and Kunze, 1994). Interestingly, since calcium permeation does not appear to be required for excitation of the peripheral sensory endings of aorticbaroreceptors (Andresen and Kunze, 1994), this suggests a selective preferential expression of calcium channels at somal membranes.

One of the key steps in synaptic transmission is the entry of calcium into the presynaptic bouton which, in turn, mediates exocytotic release of neurotransmitter. Since this presynaptic calcium step can be a key site for modulation, we utilized a series of specific antagonists to determine which calcium channels are involved in excitatory sensory afferent synaptic transmission in mNTS. Sequential addition of L-type (nimodipine), P-type (ω-agatoxin IVA) and N-type (ω-conotoxin GVIA) calcium channel antagonists (Figure 3)
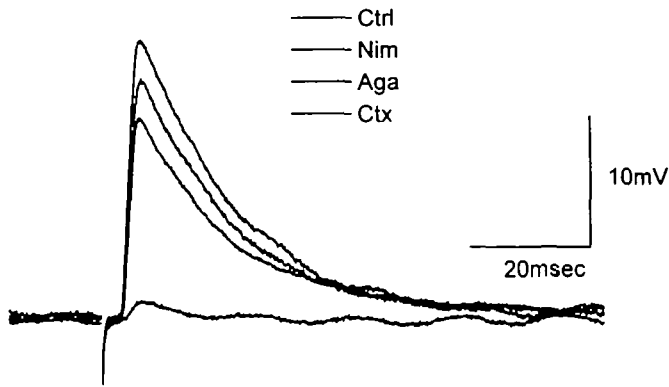
Figure 3 Calcium channel block of short-latency, EPSP from ST activation in mNTS. Ctrl is the control and uppermost trace. Antagonists were added consecutively and cumulatively: Nim (1 µM nimodipine, L-type), Aga (500 nM agatoxin, P-type) and Ctx (1 µM -conotoxin GVIA, N-type) calcium channel antagonists. Note the largest component was always Ctx sensitive and a finite response always remained in the presence of all three antagonists indicating a resistant component.
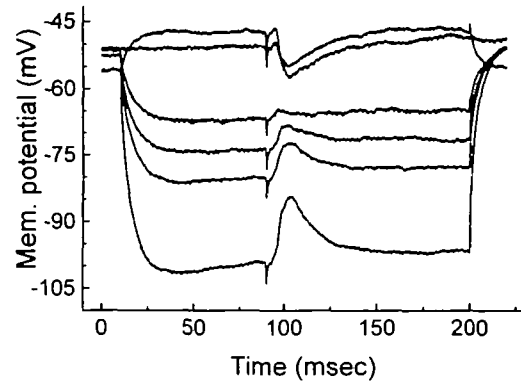

Figure 4 Long-latency inhibitory post-synaptic potential evoked by ST activation reversed to depolarizing at approximately −65 mV. This response was blocked by picrotoxin and is consistent with a GABA-A mediated increase in chloride conductance.

produced a graded depression of the ST-evoked EPSP. Sensory afferent glutamate release thus depends on at least four different presynaptic calcium channels in NTS with N-type predominating. This profile of presynaptic calcium channels in NTS is also present at the peripheral soma of the sensory neurons, but is absent from the baroreceptorsensory endings (Mendelowitz et al., 1995).

GABA is a major CNS inhibitory transmitter and has been implicated in cardiovascular regulation at caudal NTS, but this has been controversial (Van Giersbergen et al., 1992). GABA$_A$ receptors are generally post-synaptic and increase chloride conductance (Figure 4), while GABA$_B$ receptors are generally presynaptic and inhibit transmitter release by reducing calcium conductance and/or by increasing potassium conductance (Bowery, 1989; Nicoll et al., 1990). Both GABA$_A$ and GABA$_B$ binding are found in NTS (Van Giersbergen et al., 1992) and affect BP control. GABA micro-injection into NTS increases BP and GABA$_A$ antagonist blocks these increases and enhances responses to ADN stimulation (Bousquet et al., 1982; Kubo and Kihara, 1988b). Micro-injection of GABA onto NTS neurons inhibits their activation by CSN stimulation and this inhibitory effect is blocked by a GABA$_A$ antagonist (McWilliam and Shepheard, 1988). GABA$_A$ antagonist injected into NTS also reduces hypothalamic inhibition in NTS (Andresen and Kunze, 1994). Blockade of GABA uptake increases resting BP, but this inhibition of apparently ongoing release of GABA is mediated by GABA$_B$ receptors (Sved and Sved, 1990). GABA$_B$ agonist, baclofen, injected into NTS increases

BP and depresses baroreflex responses (Andresen and Kunze, 1994). Baclofen in medullary slices:

(i) depresses spontaneous and ST-evoked EPSPs and IPSPs presumably presynaptically;

(ii) directly hyperpolarizes NTS neurons (Brooks et al., 1992).

Thus, GABA$_B$ may operate by presynaptic modulation of both Glu and GABA release in NTS. Recent electron microscopic studies, however, failed to resolve presynaptic GABA terminals (Izzo et al., 1992). Thus, there is considerable inconsistency between the views of the roles of GABA$_A$ and GABA$_B$ in NTS and in all probability both mechanisms exist, possibly on different neurons.

Isolated IPSPs in mNTS which are evoked by ST stimulation occur at latencies which are typically double those encountered with EPSPs. Although the blockade of these IPSPs by picrotoxin and bicuculline, and their reversal potentials suggest that they are typical GABA-A type responses, the non-NMDA antagonist, CNQX, also blocks these IPSPs (Andresen and Yang, 1995a). Together, these observations are consistent with inhibitory neurons strongly driven by primary afferent input from ST via a two neuron local circuit.

Many peptides are associated with the sensory neurons innervating NTS and several modulate glutamatergic transmission in mNTS. For example, Angiotensin II (AII) is an important modulator within the CNS (Moffett et al., 1987; Phillips, 1987), strongly influences BP control and fluid homeostasis, and has been implicated in hypertension (Phillips, 1987). NTS contains high densities of high affinity AII receptors (Mendelsohn et al., 1984; Wamsley et al., 1990) which appear to be substantially presynaptic (Diz

et al., 1986). NTS AII levels and receptor expression are higher in SHRs than in WKY (Meyer *et al.,* 1990; Raizada *et al.,* 1993). Micro-injection of high doses of AII (50–250 ng) into NTS evokes pressor responses (Phillips, 1987). Injection of saralasin antagonist into NTS increases reflex bradycardia to increases in BP (Campagnole-Santos *et al.,* 1988), an effect consistent with inhibition of baroreflexes by endogenous AII in NTS. However, much lower doses of AII (0.1–12.5 ng) in mNTS produce depressor responses (Campagnole-Santos *et al.,* 1989; Kubo and Kihara, 1990). In horizontal slices, AII increases extracellular spike frequencies of medial NTS units (Barnes *et al.,* 1990, 1993). In medial NTS, short latency ST-NTS EPSPs were facilitated by AII without altering post-synaptic conductance or membrane potential (Yang and Andresen, 1991). How can these single NTS neuron findings be reconciled with baroreflex inhibition observed in BP data? AII inhibition of the baroreflex under these conditions may not occur at the sensory synapse or on cells directly connected to ST. Possible alternatives include AII actions at neurons beyond the first synapse. There appears to be a common framework of synaptic and cellular mechanisms in NTS and peptides may play a critical role modulating this framework to regulate overall reflex function.

Frequency-dependent depression appears to be a common feature in the NTS and other nearby regions of the medulla in response to afferent stimulation (e.g. Sessle, 1973a, b). Early work in anesthetized cats suggested that responses to electrical activation of the afferent inputs were greatly attenuated at frequencies greater than 5 Hz at sensory afferent synapses within mNTS (Seller and Illert, 1969). Afferent-NTS synaptic responses recorded intracellularly are depressed at similar frequencies *in vivo* (Mifflin and Felder, 1988, 1990) and in brain slices (Miles, 1986; Andresen and Yang, 1995a). Frequency-dependent limits of baroreflex responses to ADN stimulation may be more modest in rats, however (Gordon and Mark, 1984; Kubo and Kihara, 1988a; Reynolds and Andresen, 1993).

Clearly, a broad range of information on the nature and mechanisms of cellular function of NTS is not yet available. Fairly systematic studies will be required to examine many critical issues in NTS in order to approach the complexities of overall performance mechanisms such as the frequency transmission within NTS. Major aspects of local circuitry (secondary neurons, interneurons and output neurons) need to be defined, and studied both in isolation and within an integrative framework.

ACKNOWLEDGEMENTS

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