Published in final edited form as:

Respir Physiol Neurobiol. 2008 December 10; 164(1-2): 105–111. doi:10.1016/j.resp.2008.04.013.

Plasticity in Glutamatergic NTS Neurotransmission

David D. Kline

Department of Biomedical Sciences and Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65211

Abstract

Changes in the physiological state of an animal or human can result in alterations in the cardiovascular and respiratory system in order to maintain homeostasis. Accordingly, the cardiovascular and respiratory systems are not static but readily adapt under a variety of circumstances. The same can be said for the brainstem circuits that control these systems. The nucleus tractus solitarius (NTS) is the central integration site of baroreceptor and chemoreceptor sensory afferent fibers. This central nucleus, and in particular the synapse between the sensory afferent and second-order NTS cell, possesses a remarkable degree of plasticity in response to a variety of stimuli, both acute and chronic. This brief review is intended to describe the plasticity observed in the NTS as well as the locus and mechanisms as they are currently understood. The functional consequence of NTS plasticity is also discussed.

Keywords

synaptic plasticity; NTS; respiration; blood pressure

1. Introduction

Increasingly, studies are demonstrating that the central respiratory and cardiovascular control systems are not static, but rather undergo adaptive changes on several time scales (see Powell et al., 1998). Such adaptations may occur along one or more neural pathways within the central cardiorespiratory system. While the mechanisms, time frames and sites of adaptation may differ, the end response may ultimately alter baseline cardiorespiratory parameters and its related reflexes. In the following review, I will focus on the afferent sensory pathways of the baroreceptor and chemoreceptor reflexes that travel from the periphery to the central nervous system. Special attention will be paid to the nucleus tractus solitarius (NTS) and the synaptic signaling between visceral sensory afferents and second-order NTS cells. I will also discuss how plasticity in this circuit may contribute to acute and chronic changes in the cardiovascular and respiratory systems.

2. Nucleus Tractus Solitarius

The NTS has been the subject of several excellent reviews (van Giersbergen et al., 1992; Andresen and Kunze, 1994; Andresen et al., 2004) and therefore only a brief introduction will

---

Address for Correspondence: David D. Kline, Ph.D., Department of Biomedical Sciences, Dalton Cardiovascular Research Center, University of Missouri, 134 Research Park Dr., Columbia, MO 65211, Tel: 573-884-0505, Fax: 573-884-4232, E-mail: klinedd@missouri.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

be presented here. The NTS is located in the dorsal brainstem and is the primary site for termination and integration of peripheral sensory afferents, including baroreceptor, chemoreceptor, lung and gastrointestinal fibers. The sensory afferents enter the brainstem, travel collectively along the tractus solitarius (TS), exit the tract, and form synapses with one or more neurons in the NTS in a loose viscerotopic organization. Baroreceptor afferents typically terminate within the medial subnucleus of the NTS, while carotid body chemoreceptor afferents terminate within the medial and commissural subnucleus (Andresen and Kunze, 1994).

Glutamate is the primary neurotransmitter released from the sensory afferents (Talman et al., 1980). Glutamate binds to excitatory postsynaptic non-NMDA (N-methyl-D-aspartic acid) receptors to initiate information transfer to the NTS cell (Andresen and Yang, 1990). However, NMDA receptors may also play a prominent role in excitatory synaptic transmission (Aylwin et al., 1997), especially during elevated levels of afferent activity (Bonham and Chen, 2002). Subsequently NTS neurons project to several areas within the forebrain, brainstem and spinal cord to modulate the cardiovascular and respiratory system.

As the first site of information processing for cardiorespiratory reflexes, it is not unexpected that the NTS is also the first site where such processing can be modulated. The remarkable ability of this synapse to undergo short- and long-term plasticity in its function is an important step in bestowing reliability and adaptability in the cardiorespiratory system and its associated reflexes. Understanding the fundamental properties of the sensory afferent-NTS cell synapse and how it may adapt to a variety of stimuli, both acute and chronic, may also lead to a greater understanding of cardiorespiratory physiology in health and disease.

3. Synaptic Plasticity

Most synapses, including those in the NTS, are subject to changes in synaptic efficacy, which is defined as the ability of a presynaptic input to influence postsynaptic output (Lopez, 2002). Plasticity in synaptic transmission could occur on a short- and/or long-term time scale. Short-term plasticity, which may occur from milliseconds to many seconds, may result in a state of synaptic transmission that quickly and reversibly becomes enhanced or attenuated. Long-term plasticity may persist for hours or even days under certain conditions, resulting in prolonged alterations in synaptic transmission. Yet, whether plasticity occurs on a short- or long-term time scale, the adaptations that result will ultimately alter individual intrinsic cellular properties and likely the embedded neural network. Synaptic plasticity may augment or attenuate the net output from the NTS to downstream cardiovascular and/or respiratory nuclei. For instance, short-term plasticity events in the NTS may augment or reduce baro- and chemoreflex gain and affect blood pressure or breathing. Long-term plasticity events may ultimately alter baseline cardiovascular and respiratory parameters and set blood pressure, sympathetic nerve activity, heart rate, respiratory rate or volume at a new set point.

4. Short-term Synaptic Plasticity

4.1. Synaptic depression

Two common forms of short-term plasticity observed in the NTS are time-dependent depression (also called accommodation, Zhou et al., 1997) and frequency-dependent depression. Time-dependent depression occurs when the number of evoked action or synaptic potentials progressively decreases over time despite a fixed rate of stimulation, bringing about a lower but steady activity level. Frequency-dependent depression is observed when an increase in the frequency of stimulation leads to a greater depression in synaptic potentials or action potential discharge. In other words, an increase in afferent stimulation frequency results in a

lower steady state output. These forms of synaptic plasticity have been observed in both *in vivo* and *in vitro* preparations.

Seller and Illert (1969) in their study of extracellular potentials in the cat NTS, were one of the first to demonstrate that when the frequency of carotid sinus nerve stimulation is increased, the amplitude of the postsynaptic response progressively decreased. Depression occurred at stimulation frequencies as low as 5 Hz. Short-term depression of activity at frequencies greater than 20 Hz coincided with a reduced arterial depressor response when compared to the initial stimulus. To rule out the influence of afferent nerve failure, these authors demonstrated that compound activity of the intracranial root of the afferent glossopharyngeal nerve was unaltered at frequencies as high as 120 Hz. In contrast to afferent stimulation, direct stimulation of the NTS did not attenuate the depressor responses. This suggested that the synapse between baroreceptor sensory afferent and the NTS cell is the site of the depression and changes in its function may play a significant role in blood pressure control. Whether synaptic depression occurred as a result of presynaptic or postsynaptic alterations, or a mixture of both, was not known.

Since these initial studies, the site and mechanism of such frequency-dependent depression have been extensively studied. Mifflin and Felder (1988), in their recording of intracellular synaptic and action potentials in rats *in vivo*, demonstrated that time- and frequency-dependent depression occurred in electrophysiologically identified NTS cells that received direct sensory afferent connections (monosynaptic) from the carotid sinus nerve. When a conditioning stimulus preceded a test stimulus (similar to paired pulse ratio tests, see below), a reduction in excitatory postsynaptic potential (EPSP) amplitude was observed in the test stimulus. When the conditioning pulse was a stimulus train, the depression was even greater. Depression was seen in the absence of membrane potential or input resistance changes, suggesting it occurs at the synapse and in particular at the presynaptic terminal.

Liu et al. (2000) demonstrated in rats *in vivo* that time- and frequency-dependent depression of action potential discharge occurs in NTS cells that receive either direct (monosynaptic) or indirect (polysynaptic) connections from the aortic depressor nerve. The magnitude of depression was greater in cells receiving polysynaptic inputs. When the aortic depressor nerve was stimulated in a physiological phasic or bursting pattern, discharge depression was even greater compared to those with constant stimulation. Using curve analysis, these authors indicated that the function of frequency-dependent depression was to attenuate the magnitude of sympathoinhibition evoked by baroreflex activation and suggested such intrinsic mechanisms will “dampen excessive fluctuations in blood pressure” (Liu et al., 2000). Because time- and frequency-dependent depression occurs in NTS cells directly connected to sensory afferents, this indicates that the first synapse (and thus the second-order neuron) does not simply act as a relay station. Given the importance of the NTS as a major autonomic integration center, it is not surprising that it plays an important role in signal integration. Taken together, these *in vivo* studies demonstrate significant short-term plasticity occurs at the sensory afferent-NTS cell synapse and modulates reflex output. Further, the application of computational modeling approaches and single cell studies in brain slices is providing a better understanding of the locus and mechanisms underlying these short-term plasticity events.

Brain slices have extensively been used to determine the mechanism(s) of time- and frequency-dependent depression in the NTS. They contain all, or a portion, of the TS and the NTS cells to which sensory afferents form synaptic contact. When used in conjunction with fluorescent tracer labeling of the afferent fibers, these reduced slice preparations allow examination of a specific NTS cell population, i.e., cells receiving baroreceptor, chemoreceptor or lung afferents (Doyle and Andresen, 2001; Bonham and Chen, 2002; Kline et al., 2002). Moreover, retrograde labeling techniques have allowed the identification and direct electrophysiological study of

NTS output neurons (Haddad and Getting, 1989; Bailey et al., 2006). Slice preparations also allow detailed analysis of intrinsic membrane and synaptic properties, their modulation by various neurotransmitter/modulatory systems as well as the mechanisms underlying this plasticity.

In the NTS, data obtained from slices suggest that the sensory afferent-NTS synapse is a strong connection and subject to immense neuromodulation (Andresen et al., 2004). In the majority of NTS cells that receive direct (monosynaptic) connections from sensory afferents, single TS shocks elicit large amplitude, relatively invariant excitatory postsynaptic currents (EPSCs) which is indicative of a synapse with a high probability of release (Doyle and Andresen, 2001; Kline et al., 2002; Andresen et al., 2004; Kline et al., 2007). However, as *in vivo*, successive TS stimulation dramatically reduces postsynaptic current or potential amplitude compared to the first event (time-dependent depression, or accommodation). Furthermore, as the frequency of stimulation increases, so does the magnitude of EPSC depression (frequency-dependent depression). Thus, as *in vivo*, short-term plasticity is observed at the *in vitro* NTS first synapse.

Miles (1986) was one of the first to demonstrate time- and frequency-dependent depression in NTS postsynaptic cells using an *in vitro* guinea pig NTS preparation. In his preparation, repeatedly stimulating the TS at a given frequency resulted in EPSP accommodation with no change in postsynaptic membrane potential. TS stimulation at progressively higher frequencies further decreased EPSPs to a new steady state. For instance, at 1 Hz, amplitude was reduced to 91% of control (taken at 0.5 Hz) and at 5, 10 and 20 Hz, EPSPs were further reduced to 63%, 39% and 17% of control respectively. Later, Andresen and Yang (1990; 1995) demonstrated non-NMDA receptors are the primarily initiators of synaptic transmission in the medial NTS and that time- and frequency-dependent depression occurred in monosynaptic cells in the horizontal preparation of the rat NTS. Comparing the depression evoked by phasic TS stimuli to that from tonic TS stimuli, these authors demonstrated that while both stimuli elicited synaptic depression, in a third of NTS cells studied the more physiological phasic pattern resulted in less depression and was more effective in eliciting action potentials. This group has recently observed frequency-dependent depression in enhanced green fluorescent protein (EGFP)-identified GABAergic inhibitory (Bailey et al., 2008) and catecholaminergic (Appleyard et al., 2007) cells. Chen et al. (1999) demonstrated frequency-dependent depression occurred in both monosynaptic and polysynaptic cell in the rat *in vitro* NTS, and that AMPA and NMDA receptor components were equally depressed. Zhou et al. (1997) demonstrated that prolonged low-frequency (5 Hz) stimulation of the TS produced synaptic accommodation and in some cells depression continued for more than 30 minutes. The latter is similar to the long-term depression (LTD) observed in slices from other brain regions (Massey and Bashir, 2007). Time and frequency-dependent depression was also observed in the NTS using field potential analysis (Zhou and Poon, 2000).

Taken together, *in vivo* and *in vitro* studies demonstrate that moderate levels of afferent stimulation depress synaptic transmission (accommodation or time-dependent depression) and this increases with higher frequency (frequency-dependent depression). The profound depression observed with higher stimulus frequencies suggests the first synapse in the NTS acts as a low pass filter, allowing low frequency stimuli to be transmitted to the NTS with greater fidelity compared to higher frequency stimuli. Interestingly, synaptic depression is sometimes observed at frequencies as low as 1 Hz, a level which is well below the maximum discharge observed in baroreceptor A-type (80 Hz) and C-type (20 Hz) fibers (Andresen and Kunze, 1994) and chemoreceptor fibers (20 Hz) (Vidruk et al., 2001). Altogether, this suggests such acute synaptic depression is commonplace in NTS neurons of several phenotypes and likely plays a physiological role. Although the exact functional role of synaptic depression at this first synapse in reflex function or cardiorespiratory homeostasis remains under

investigation, plasticity may result in efficient processing of sensory afferent information in NTS cells and proper functioning of autonomic reflexes.

4.1.1. Potential mechanisms of short-term depression—Several possible mechanisms have been suspected as mediating short-term plasticity in the NTS. These include: changes in neurotransmitter vesicle release due to intrinsic properties or intracellular signaling cascades, an imbalance between excitatory and inhibitory neuromodulators or neurotransmitters, alterations in ion channel activity that modulate presynaptic or postsynaptic intrinsic excitability, modulation of postsynaptic glutamate receptor sensitivity, slow transmitter vesicle turnover following release, or a mixture of the all of the above.

Short-term synaptic depression is primarily believed to be due to a presynaptic mechanism. Miles (1986) in the studies described above suggested depression was due to presynaptic vesicle depletion rather than changes in the excitability of the postsynaptic neuron. Chen et al. (1999) have also demonstrated synaptic depression is due to presynaptic mechanisms. Examining synaptic transmission at several stimulation frequencies in the presence of cyclothiazide, an AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor desensitization blocker, they showed frequency-dependent synaptic depression continued at stimulation frequencies up to 24 Hz. Such data suggest postsynaptic receptor desensitization was not the primary mechanism for synaptic depression. Here, as in all studies using cyclothiazide, it is important to note that it may also facilitate neurotransmitter release by inhibiting presynaptic potassium conductance as well as its direct effects on exocytotic machinery (Ishikawa and Takahashi, 2001).

The roles of many neurotransmitters and modulators have been examined in synaptic neurotransmission and time- and frequency-dependent plasticity in the NTS. Angiotensin II (Barnes et al., 2003), substance P (Sekizawa et al., 2003; Bailey et al., 2004), enkephalins (Glatzer and Smith, 2005), adenosine/ATP (Kato and Shigetomi, 2001; Shigetomi and Kato, 2004), prostaglandin E2 (Laaris and Weinreich, 2007) and norepinephrine (Zhang and Mifflin, 2007) alter neurotransmitter release, postsynaptic activity, or the magnitude of synaptic depression. Dopamine also reduces TS-evoked glutamate release to induce synaptic depression in the NTS (Kline et al., 2002). This occurred in cells directly apposed with fluorescently-tagged chemoreceptor and vagal afferent boutons. Dopamine-mediated synaptic depression was due primarily to activation of presynaptic D2-like receptors rather than D1-like receptors. Interestingly blockade of D2 receptors increased synaptic transmission, suggesting that dopamine is tonically released from chemoreceptor and vagal afferents to persistently attenuate neurotransmission.

In other central nuclei, dopamine's presynaptic action occurs through changes in ionic currents or synaptic vesicle proteins. D2 receptors may attenuate calcium entry through N-type channels, the primary mediators of calcium-induced vesicle release in NTS sensory afferents (Mendelowitz et al., 1995), to reduce synaptic transmission (Missale et al., 1998; Momiyama and Koga, 2001; Lisman et al., 2007). Dopamine may also modulate the functional state of one or more synaptic vesicle proteins, such as the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) core complex. This synaptic protein complex consists of three proteins important for vesicle exocytosis: VAMP (vesicle associated membrane protein, also called synaptobrevin); syntaxin; and SNAP25 (synaptosomal associated protein of 25kDa (Lisman et al., 2007)). Following release of vesicle contents, the SNARE complex is recycled so that individual components can take part in another round of membrane fusion. Fisher and Braun (2000) demonstrated DA increases SNARE complex formation; which may reduce vesicle recycling and the number of vesicles available for release during extended stimulus trains. Modeling studies have suggested a similar mechanism plays an important role in synaptic transmission in the NTS (see below).

While glutamate is the primary excitatory neurotransmitter acting on postsynaptic non-NMDA receptors, it also binds to presynaptic metabotropic receptors to induce depression. (Glaum and Miller 1992; 1993a; 1993b) demonstrated that activation of presynaptic metabotropic receptors decreases excitatory synaptic neurotransmission in the rat transverse *in vitro* slice. In their studies, synaptic depression was achieved through metabotropic receptor-induced inhibition of presynaptic N-type calcium channels (Glaum and Miller, 1995). Activation of presynaptic Type II and III metabotropic receptors presumably contributed to the frequency-dependent depression observed in *in vitro* slices, as blockade of these receptors attenuated synaptic depression (Chen et al., 2002). Blockade of metabotropic receptors also attenuated depression of action potential discharge in response to afferent stimulation *in vivo* (Liu et al., 1998). However, the actions of metabotropic glutamate receptors in the NTS are complex as postsynaptic metabotropic receptors are excitatory and increase EPSP amplitude or action potential discharge (Glaum and Miller, 1993a; Sekizawa and Bonham, 2006).

Taken together, the aforementioned studies suggest a multitude of transmitters and modulators can modulate synaptic transmission and short-term plasticity in the NTS. It is therefore likely that depression is not due to a single neurotransmitter or modulator, but rather a complex combination of inhibitory and excitatory influences. Possibly, the multitude of neurotransmitter/modulators that influence synaptic transmission allow a variety of messages to be sent to the NTS. Many neurochemicals may affect a common signaling pathway, such as ion channels or second messengers, to modulate pre- and postsynaptic membrane potential, intracellular calcium, or synaptic vesicle proteins responsible for release.

Glutamate release depends upon the activation of presynaptic calcium channels. As discussed above, the L-, N- and P/Q-type calcium channels are high-voltage activated channels that are dominant on presynaptic nerve terminals, including those of the NTS (Mendelowitz et al., 1995; Meir et al., 1999), and interact directly with the presynaptic exocytotic machinery. Blockade of N-type calcium channels reduces or eliminates neurotransmitter release in the NTS, with P- and L-type channels playing a smaller role (Mendelowitz et al., 1995). Because of the small size of the sensory afferent terminal (< 2 μ m) in the NTS, it is technically difficult to record terminal calcium currents and determine their role in synaptic depression. However, the large presynaptic terminal of the calyx of Held in the medial nucleus of the trapezoid body allows direct recording of presynaptic calcium currents. Studies in this synapse indicate that regulation of voltage-gated calcium channels can significantly modulate short-term plasticity. A decrease in presynaptic calcium current occurs during repetitive action potential stimulation at frequencies between 2 and 30 Hz and induces synaptic depression (Xu and Wu, 2005). Whether such activity-dependent inactivation of calcium currents occurs at similar frequencies in sensory afferent terminals in the NTS to induce synaptic depression remains a compelling question.

In contrast to calcium's short-term presynaptic effect on neurotransmission, persistent accumulation of postsynaptic calcium modulates the long lasting synaptic depression observed in some NTS cells for 30 minutes or more (i.e., LTD, Zhou et al., 1997). In these studies, LTD was blocked by chelation of postsynaptic calcium by EGTA or NMDA receptor blockade. Also of note, blockade of AMPA receptor desensitization with cyclothiazide also abolished LTD, but not the accommodation or time-dependent depression.

Potassium channels may affect neurotransmitter release because of their ability to set the resting membrane potential, influence action potential duration and interspike intervals, and terminate periods of activity (Pongs, 1999; Hille, 2001). Changes in activation or inactivation properties of an individual or group of potassium channels may either hyperpolarize or depolarize terminal potential, affect calcium channel activation and subsequently alter neurotransmitter release. Microinjection of dendrotoxin, a blocker of several potassium channels, and 4-AP, a general

potassium channel blocker, into the NTS alters the baroreceptor and cardiopulmonary reflexes in the intact animal, confirming their role as mediators of cellular activity (Butcher and Paton, 1998).

We have previously examined the role of Kv1.1, a member of the Shaker-like family of potassium channels, on NTS synaptic transmission and respiration in the mouse. This delayed rectifier channel activates upon depolarization to terminate neuronal activity (Hille, 2001). Utilizing a knockout mouse lacking Kv1.1, we examined the role of these potassium channels on synaptic transmission and frequency-dependent depression (Kline et al., 2005). Analyzing EPSC amplitude at 5 and 10 Hz (compared to 0.5 Hz), frequency-dependent depression tended to be less in Kv1.1 null mice compared to littermate controls. For instance, EPSC amplitude at 5 and 10 Hz decreased in control mice to 67 and 63% of 0.5 Hz baseline, whereas in Kv1.1 mice, EPSCs decreased to only 78 and 72% of baseline. In addition to reduced depression, Kv1.1 deletion increased miniature EPSCs (events recorded in tetrodotoxin and independent of activity) and asynchronous EPSCs (release events that occur out of synchrony with the stimulus) following a physiological stimulus train. Asynchronous release is believed to occur through an increase in presynaptic residual calcium and contribute to smooth synaptic signaling (Lu and Trussell, 2000). This synaptic plasticity in the chemosensory-NTS circuit coincided with an augmentation in the respiratory response to hypoxia, the chemoreflex, in knockout mice.

Alternatively, synaptic plasticity may occur through the changes in concentration of neurotransmitter in the synaptic cleft or alterations in the intrinsic properties of the synaptic cycle, (i.e., changes in vesicle binding, release or turnover). Transmitter concentration in the cleft is governed by diffusion and uptake via various transporters, which have been suggested to play a prominent role in plasticity in other systems (Tzingounis and Wadiche, 2007) but are relatively unexplored in the NTS. However, Chen and Bonham (2005) recently demonstrated glutamate spillover at the NTS synapse can activate metabotropic glutamate receptors located on GABA terminals to decrease GABA release. Endogenous glutamate transporters influence the magnitude of GABA release. Second messenger pathways induced by receptor activation or activity-induced changes may also modulate vesicle release or turnover. For instance, the phosphorylation state of one or more synaptic proteins, such as the SNARE complex, can increase or decrease the binding of transmitter vesicles to the membrane or the movement of vesicles from the reserve pool to the readily releasable pool (Lisman et al., 2007). Recent data suggests the vesicle pools responsible for asynchronous and synchronous release may be separate, adding an additional level of plasticity in the NTS (Sara et al., 2005; Kline et al., 2007). Through a series of experimental and modeling studies, Schild and colleagues (1995) demonstrated that as the frequency of stimulation increases, frequency- and time-dependent depression is dominated primarily by the mobilization of synaptic vesicles and their refilling, with depression occurring due to vesicle depletion. Such modeling studies in the NTS clearly bring to light many mechanisms that are difficult to evaluate experimentally.

4.2 Synaptic Facilitation

In contrast to short-term synaptic depression in the NTS, synaptic facilitation has not been seen as frequently. Mifflin (1997) demonstrated in intact rats that two minutes of 100–300 Hz stimulation of the carotid sinus, aortic or vagus nerve augmented monosynaptic and polysynaptic EPSPs and action potential discharge. Likewise, in *in vitro* slices, high frequency afferent stimulation increased synaptic strength when recorded with either sharp electrode (Fortin et al., 1992) or whole cell patch electrodes (Glaum and Miller, 1993b; Zhou et al., 1997).

The mechanism involved with postsynaptic potentiation in the NTS has not been extensively studied. In other central neurons, such enhancement of synaptic transmission is thought to be

induced through a residual increase in presynaptic calcium concentrations. Additional studies, however, are required to determine whether a similar mechanism occurs in the NTS. Additionally, the molecular “switch” that converts synaptic depression to synaptic potentiation requires identification. The physiological role of experimentally-induced posttetanic potentiation also remains an interesting question, especially when one considers the frequency used to induce it relative to the normal discharge of A- and C-type sensory afferents. It is conceivable that the convergence of several sensory afferent fibers onto a NTS cell could result in these cells receiving such elevated activity.

4.3 Summary

Short-term synaptic plasticity (depression and to a lesser extent facilitation) is readily observed in the NTS. One or more mechanisms are apparently responsible for such intrinsic depression. Additionally, the magnitude of synaptic depression can be modulated by additional factors. As new techniques are developed and utilized in the NTS, the mechanism of short-term plasticity and its physiological role may soon be understood.

5. Long-term plasticity in the NTS glutamatergic synapse

Long-term synaptic plasticity in the NTS has been studied following the induction of pathophysiological or environmental conditions, including hypertension, chronic ozone or allergen exposure, and chronic sustained and intermittent hypoxia. It is important to note that in the context of this review, long-term plasticity pertains to the changes in synaptic transmission that occur over hours to days. This is in contrast to other central nuclei, such as the hippocampus, where long-term plasticity events like potentiation or depression occur over minutes to hours.

In a unique, chronic *in vivo* NTS rat preparation, Tang and Dworkin (2007) showed that potentiation of NTS activity can occur over several hours following afferent stimulation. In their studies, high frequency (100 Hz) stimulation of A- and C-fibers of the aortic depressor nerve can induce a 10 hour potentiation of extracellular discharge in response to A-type fiber stimulation. In one rat, synaptic potentiation was blocked by a NMDA receptor antagonist.

Following the induction of hypertension, there is a blunting of the baroreflex, changes occur in the properties of NTS neurons, and integration and responsiveness of NTS neurons to aortic nerve inputs are altered. Zhang and Mifflin (2000) demonstrated that when hypertension is induced in the one kidney, renal wrap model, aortic nerve stimulation increases action potential discharge in NTS cells connected by more than one synapse to sensory afferents (i.e., polysynaptic). Potentiation did not occur in monosynaptic NTS neurons. The repetitively firing polysynaptically connected neurons also made up a greater percentage of cells recorded in hypertensive animals, suggesting possible functional, timing and/or structural changes of aortic afferent fibers or their synapses. These authors suggest that the shift to greater activity in polysynaptic NTS neurons, a portion of which are inhibitory GABAergic cells, may contribute to the reduced aortic nerve depressor responses in hypertension (Zhang and Mifflin, 2000). Although in these studies it is difficult to differentiate the presynaptic and postsynaptic locus of such changes, Mifflin's laboratory has demonstrated several postsynaptic alterations. For instance, in isolated NTS cells receiving aortic depressor nerve input, hypertension (induced as above) reduced transient A- type potassium currents (Belugin and Mifflin, 2005) and increased high voltage-activated calcium currents (Tolstykh et al., 2007). The functional role of these ion channel alterations on synaptic transmission and the reflex as a whole in hypertensive rats remains an intriguing question.

While it is unknown if synaptic plasticity at NTS synapses occurs following hypertension, AMPA receptor mRNA and protein levels increase in NTS neurons of spontaneously

hypertensive rats (SHR) compared to WKY normotensive rats (Aicher et al., 2003; Saha et al., 2004), although not all studies agree (Ashworth-Preece et al., 1999). Utilizing the DOCA-salt sensitive rat model of hypertension, Hermes et al (2008) identified increased levels of GluR1 receptor subunit of the AMPA receptor in second-order vagal NTS neurons. The increase in the GluR1 subunit expression reversed when arterial pressure returned to normal with the vasodilator hydralazine. Hydralazine also reduced GluR1 subunit expression in the NTS in SHR's. Interestingly, NTS cells in hypertensive rats showed increased numbers of dendritic spines, perforated synapses that are related to activity-dependent plasticity such as hippocampal LTP, and glial ensheathing. Thus, hypertension induces structural plasticity at the NTS glutamatergic synapse. Similarly, an acute elevation in arterial pressure induced structural plasticity in NTS cells in as little as 2 hours as evidenced by increases in nuclear membrane invagination which is suggestive of increased transcription (Chan et al., 2000). It is tempting to speculate that such transcription is relevant to postsynaptic receptors; however, there is no evidence to support this notion. Nonetheless, these increases in AMPA receptor subunit expression and structural changes at the synapse suggest alterations in neurotransmission may occur at the afferent-NTS synapse.

Plasticity of synaptic and neuronal activity following chronic ozone or allergen exposure has also been studied. In a study utilizing brainstem slices from young primates, 14 days of ozone exposure induced synaptic plasticity when compared to filtered air controls (Chen et al., 2003). Ozone exposure decreased TS-evoked action potentials discharge. This was counterbalanced by increased postsynaptic discharge when induced by current injection. Ozone exposure also depolarized NTS membrane potential. Results suggest ozone exposure reduces presynaptic glutamate release while increasing postsynaptic excitability. Ozone exposure also increased the influence of substance P on NTS neuroplasticity. Blockade of substance P (NK1) receptors attenuated the elevated current-induced discharge, but did not affect the synaptic depression. Thus, ozone exposure induces neuroplasticity in the NTS, and this neuroplasticity can in turn be modified by substance P. In similar studies, chronic allergen (house dust mites) exposure in rhesus monkeys *in vivo* was associated with increased action potential discharge of NTS neurons when they were subsequently studied in brains slices (Chen et al., 2001). However, whether synaptic transmission was altered following allergen exposure is uncertain.

Our studies have established that 10 days of chronic intermittent hypoxia (CIH, a cycle of 21 and 5% inspired O₂, 8 hours per day) leads to plasticity in the afferent limb of the arterial chemoreflex of the rat (Kline et al., 2007). In particular, changes occurred in the synaptic transmission between chemosensory afferents and NTS second order cells examined in brainstem slices. CIH significantly increased baseline NTS action potential discharge and promoted action potential short-term facilitation following 20 Hz train TS stimulation. This occurred despite decreased TS-evoked synaptic transmission between chemosensory afferents and NTS cells. Further, an increase in asynchronous and miniature neurotransmitter release counter-balanced the decrease in evoked transmission and increased overall NTS neuronal transmission. Using the recorded asynchronous EPSCs as our current command, we determined that an increase in asynchronous release is sufficient to induce action potential discharge and together with the elevated miniature activity is partly responsible for short-term facilitation of action potential discharge. Further, blockade of calcium-calmodulin kinase II (CaMKII), which mediates hippocampal LTD, attenuated CIH-induced TS-evoked synaptic depression but did not affect postsynaptic properties (Kline et al., 2007). The decrease in TS-evoked EPSCs persisted following 30 days of CIH and alterations were partly restored after two weeks of only room air. Taken together, our data suggest that CIH-induced plasticity occurs through a presynaptic mechanism. The time course of its development as well as its reversibility would suggest changes in the expression or function of one or more presynaptic proteins. We did not observe a change in postsynaptic AMPA receptor properties such as miniature EPSC amplitude, AMPA receptor kinetics or desensitization. Input resistance and membrane

potential was also not changed. Altogether, alterations in postsynaptic properties do not appear to mediate CIH-induced plasticity. By contrast, de Paula (2007) demonstrated that following 7 days of CIH, dispersed NTS cells studied *in vitro* exhibit enhanced postsynaptic AMPA and NMDA receptor currents to brief agonist application. These authors also showed that AMPA receptor desensitization was not altered in CIH. The differences between these latter two CIH studies in the NTS may be due to the differing preparations (slices versus isolated cells), the duration of individual hypoxic or normoxic exposure, the fraction of inspired O₂ used during each level of hypoxia, or the duration (days) of the CIH exposure. Recent results from isolated cells suggest the latter may play an important role in synaptic plasticity (see below). Such time-dependent changes in synaptic transmission in CIH are in agreement with the concept that the pattern and duration of a hypoxic exposure may significantly modulate cardiorespiratory outcome (Prabhakar and Kline, 2002).

5.1 Relevance

The above described plasticity in synaptic transmission and cellular neuroexcitability induced by chronic stimulation is consistent with the notion of homeostatic plasticity and activity-dependent changes in neuronal function in other central neurons (Davis and Bezprozvanny, 2001; Turrigiano, 2007). Homeostatic plasticity is a form of plasticity that is thought to stabilize cellular activity in the face of persistent changes in excitability. As discussed by Turrigiano (2007), induction of homeostatic plasticity requires that neurons can sense cellular activity, integrate this activity, and compare it to a given set point. Subsequent adjustments in cellular and synaptic properties bring neuronal activity back to the set point. As discussed, these properties are evident in the NTS.

Homeostatic plasticity has best been described in dissociated cell preparations from other central nuclei where neuronal activity is most easily manipulated in culture. For instance, the addition of tetrodotoxin to the culture medium will decrease cellular and network activity of cultured neurons. Conversely, bicuculline in the medium will block GABA mediated inhibition and increase cellular and network activity. *In vitro* cortical synapses modulated postsynaptic activity in response to changes in presynaptic excitation. An increase in activity decreased quantal size whereas decreasing activity increased quantal size. This occurs through insertion of postsynaptic receptor to the membrane. On the other hand, *in vitro* studies in cultured hippocampal neurons showed increased presynaptic neurotransmitter release in response to inactivity (see Turrigiano, 2007). *In vivo* studies in the developing visual cortex of rat showed that monocular deprivation selectively increased EPSC amplitudes of pyramidal neurons of the deprived eye, yet did not alter amplitudes to the control side (Turrigiano and Nelson, 2004).

The mechanism(s) and locus of homeostatic plasticity remains under intense investigation. Many of the mechanism discussed above have been implicated. A recent study in cortical and hippocampal neurons suggested that the time spent *in vitro* is a major determinant of the plasticity locus. Activity deprivation in cultures less than 2.5 weeks old elicited a purely postsynaptic mechanism, whereas cultures older than 3 weeks showed increased quantal amplitude brought on by a mixed presynaptic and postsynaptic mechanism (Wierenga et al., 2006). Whether a similar temporal and age regulation of synaptic plasticity occurs in the NTS is a compelling question.

While the notion of homeostatic plasticity has been championed in cortical, hippocampal or visual neurons, there is no system whose sole function is to maintain bodily homeostasis other than the autonomic nervous and respiratory system. The cardiovascular and respiratory circuits serve vital functions and must remain stable, yet flexible, for the preservation of life. Maintenance of blood pressure occurs via the actions of the baroreflex to maintain cardiovascular homeostasis. Arterial blood gases are maintained by the central and peripheral

chemoreflex control of breathing. In short, homeostatic control of blood pressure and respiration is maintained throughout life despite the diversity of demands placed on these systems by normal physiological and pathophysiological processes. It is likely that synaptic plasticity in the NTS and other parts of the autonomic nervous system plays a vital role in cardiovascular and respiratory homeostasis.

Acknowledgements

The author thanks Dr. Eileen M. Hasser for helpful comments on this manuscript. The work performed and described by the author was supported while in the laboratory of Dr. Diana L. Kunze (HL-25830, DLK) and the University of Missouri.

References

- Aicher SA, Sharma S, Mitchell JL. Structural changes in AMPA-receptive neurons in the nucleus of the solitary tract of spontaneously hypertensive rats. *Hypertension* 2003;41:1246–1252. [PubMed: 12695422]
- Andresen MC, Doyle MW, Bailey TW, Jin YH. Differentiation of autonomic reflex control begins with cellular mechanisms at the first synapse within the nucleus tractus solitarius. *Braz. J. Med. Biol. Res* 2004;37:549–558. [PubMed: 15064818]
- Andresen MC, Kunze DL. Nucleus tractus solitarius--gateway to neural circulatory control. *Annu. Rev. Physiol* 1994;56:93–116. [PubMed: 7912060]
- Andresen MC, Yang M. Dynamics of sensory afferent synaptic transmission in aortic baroreceptor regions on nucleus tractus solitarius. *J. Neurophysiol* 1995;74:1518–1528. [PubMed: 8989390]
- Andresen MC, Yang MY. Non-NMDA receptors mediate sensory afferent synaptic transmission in medial nucleus tractus solitarius. *Am. J. Physiol* 1990;259:H1307–H1311. [PubMed: 1977326]
- Appleyard SM, Marks D, Kobayashi K, Okano H, Low MJ, Andresen MC. Visceral afferents directly activate catecholamine neurons in the solitary tract nucleus. *J. Neurosci* 2007;27:13292–13302. [PubMed: 18045923]
- Ashworth-Preece MA, Chen F, Jarrott B, Lawrence AJ. Visualisation of AMPA binding sites in the brain stem of normotensive and hypertensive rats. *Brain Res* 1999;834:186–189. [PubMed: 10407113]
- Aylwin ML, Horowitz JM, Bonham AC. NMDA receptors contribute to primary visceral afferent transmission in the nucleus of the solitary tract. *J. Neurophysiol* 1997;77:2539–2548. [PubMed: 9163375]
- Bailey CP, Maubach KA, Jones RS. Neurokinin-1 receptors in the rat nucleus tractus solitarius: pre- and postsynaptic modulation of glutamate and GABA release. *Neuroscience* 2004;127:467–479. [PubMed: 15262336]
- Bailey TW, Appleyard SM, Jin YH, Andresen MC. Organization and Properties of GABAergic Neurons in Solitary Tract Nucleus (NTS). *J. Neurophysiol* 2008;99:1712–1722. [PubMed: 18272881]
- Bailey TW, Hermes SM, Andresen MC, Aicher SA. Cranial visceral afferent pathways through the nucleus of the solitary tract to caudal ventrolateral medulla or paraventricular hypothalamus: target-specific synaptic reliability and convergence patterns. *J. Neurosci* 2006;26:11893–11902. [PubMed: 17108163]
- Barnes KL, DeWeese DM, Andresen MC. Angiotensin potentiates excitatory sensory synaptic transmission to medial solitary tract nucleus neurons. *Am. J. Physiol Regul. Integr. Comp Physiol* 2003;284:R1340–R1353. [PubMed: 12531785]
- Belugin S, Mifflin S. Transient voltage-dependent potassium currents are reduced in NTS neurons isolated from renal wrap hypertensive rats. *J. Neurophysiol* 2005;94:3849–3859. [PubMed: 16293589]
- Bonham AC, Chen CY. Glutamatergic neural transmission in the nucleus tractus solitarius: N-methyl-D-aspartate receptors. *Clin. Exp. Pharmacol. Physiol* 2002;29:497–502. [PubMed: 12010198]
- Butcher JW, Paton JF. K⁺ channel blockade in the NTS alters efficacy of two cardiorespiratory reflexes in vivo. *Am. J. Physiol* 1998;274:R677–R685. [PubMed: 9530233]

- Chan RK, Peto CA, Sawchenko PE. Fine structure and plasticity of barosensitive neurons in the nucleus of solitary tract. *J. Comp Neurol* 2000;422:338–351. [PubMed: 10861511]
- Chen CY, Bonham AC. Glutamate suppresses GABA release via presynaptic metabotropic glutamate receptors at baroreceptor neurones in rats. *J. Physiol* 2005;562:535–551. [PubMed: 15539399]
- Chen CY, Bonham AC, Plopper CG, Joad JP. Neuroplasticity in nucleus tractus solitarius neurons after episodic ozone exposure in infant primates. *J. Appl. Physiol* 2003;94:819–827. [PubMed: 12433861]
- Chen CY, Bonham AC, Schelegle ES, Gershwin LJ, Plopper CG, Joad JP. Extended allergen exposure in asthmatic monkeys induces neuroplasticity in nucleus tractus solitarius. *J. Allergy Clin. Immunol* 2001;108:557–562. [PubMed: 11590381]
- Chen CY, Horowitz JM, Bonham AC. A presynaptic mechanism contributes to depression of autonomic signal transmission in NTS. *Am. J. Physiol* 1999;277:H1350–H1360. [PubMed: 10516169]
- Chen CY, Ling EH, Horowitz JM, Bonham AC. Synaptic transmission in nucleus tractus solitarius is depressed by Group II and III but not Group I presynaptic metabotropic glutamate receptors in rats. *J. Physiol* 2002;538:773–786. [PubMed: 11826164]
- Davis GW, Bezprozvanny I. Maintaining the stability of neural function: a homeostatic hypothesis. *Annu. Rev. Physiol* 2001;63:847–869. [PubMed: 11181978]
- de Paula PM, Tolstykh G, Mifflin SW. Chronic intermittent hypoxia alters NMDA and AMPA-evoked currents in NTS neurons receiving carotid body chemoreceptor inputs. *Am. J. Physiol Regul. Integr. Comp Physiol*. 2007
- Doyle MW, Andresen MC. Reliability of monosynaptic sensory transmission in brain stem neurons in vitro. *J. Neurophysiol* 2001;85:2213–2223. [PubMed: 11353036]
- Fisher H, Braun JE. Modulation of the SNARE core complex by dopamine. *Can. J. Physiol Pharmacol* 2000;78:856–859. [PubMed: 11077986]
- Fortin G, Velluti JC, avit-Saubie M, Champagnat J. Responses to repetitive afferent activity of rat solitary complex neurons isolated in brainstem slices. *Neurosci. Lett* 1992;147:89–92. [PubMed: 1362263]
- Glatzer NR, Smith BN. Modulation of synaptic transmission in the rat nucleus of the solitary tract by endomorphin-1. *J. Neurophysiol* 2005;93:2530–2540. [PubMed: 15615836]
- Glaum SR, Miller RJ. Metabotropic glutamate receptors mediate excitatory transmission in the nucleus of the solitary tract. *J. Neurosci* 1992;12:2251–2258. [PubMed: 1351539]
- Glaum SR, Miller RJ. Activation of metabotropic glutamate receptors produces reciprocal regulation of ionotropic glutamate and GABA responses in the nucleus of the tractus solitarius of the rat. *J. Neurosci* 1993a;13:1636–1641. [PubMed: 7681873]
- Glaum SR, Miller RJ. Metabotropic glutamate receptors depress afferent excitatory transmission in the rat nucleus tractus solitarii. *J. Neurophysiol* 1993b;70:2669–2672. [PubMed: 7907134]
- Glaum SR, Miller RJ. Presynaptic metabotropic glutamate receptors modulate omega-conotoxin-GVIA-insensitive calcium channels in the rat medulla. *Neuropharmacology* 1995;34:953–964. [PubMed: 8532176]
- Haddad GG, Getting PA. Repetitive firing properties of neurons in the ventral region of nucleus tractus solitarius. In vitro studies in adult and neonatal rat. *J. Neurophysiol* 1989;62:1213–1224. [PubMed: 2600620]
- Hermes SM, Mitchell JL, Silverman MB, Lynch PJ, McKee BL, Bailey TW, Andresen MC, Aicher SA. Sustained hypertension increases the density of AMPA receptor subunit, GluR1, in baroreceptive regions of the nucleus tractus solitarii of the rat. *Brain Res* 2008;1187:125–136. [PubMed: 18031714]
- Hille, B. Ion channels of excitable membranes. Sunderland, MA: Sinauer Associates Inc.; 2001.
- Ishikawa T, Takahashi T. Mechanisms underlying presynaptic facilitatory effect of cyclothiazide at the calyx of Held of juvenile rats. *J. Physiol* 2001;533:423–431. [PubMed: 11389202]
- Kato F, Shigetomi E. Distinct modulation of evoked and spontaneous EPSCs by purinoceptors in the nucleus tractus solitarii of the rat. *J. Physiol* 2001;530:469–486. [PubMed: 11158277]
- Kline DD, Buniel MC, Glazebrook P, Peng YJ, Ramirez-Navarro A, Prabhakar NR, Kunze DL. Kv1.1 deletion augments the afferent hypoxic chemosensory pathway and respiration. *J. Neurosci* 2005;25:3389–3399. [PubMed: 15800194]

- Kline DD, Ramirez-Navarro A, Kunze DL. Adaptive depression in synaptic transmission in the nucleus of the solitary tract after in vivo chronic intermittent hypoxia: evidence for homeostatic plasticity. *J. Neurosci* 2007;27:4663–4673. [PubMed: 17460079]
- Kline DD, Takacs KN, Ficker E, Kunze DL. Dopamine modulates synaptic transmission in the nucleus of the solitary tract. *J. Neurophysiol* 2002;88:2736–2744. [PubMed: 12424308]
- Laaris N, Weinreich D. Prostaglandin E2 depresses solitary tract-mediated synaptic transmission in the nucleus tractus solitarius. *Neuroscience* 2007;146:792–801. [PubMed: 17367942]
- Lisman JE, Raghavachari S, Tsien RW. The sequence of events that underlie quantal transmission at central glutamatergic synapses. *Nat. Rev. Neurosci* 2007;8:597–609. [PubMed: 17637801]
- Liu Z, Chen CY, Bonham AC. Metabotropic glutamate receptors depress vagal and aortic baroreceptor signal transmission in the NTS. *Am. J. Physiol* 1998;275:H1682–H1694. [PubMed: 9815076]
- Liu Z, Chen CY, Bonham AC. Frequency limits on aortic baroreceptor input to nucleus tractus solitarius. *Am. J. Physiol Heart Circ. Physiol* 2000;278:H577–H585. [PubMed: 10666090]
- Lopez JC. Quantifying synaptic efficacy. *Nat Rev Neurosci* 2002;3:332.
- Lu T, Trussell LO. Inhibitory transmission mediated by asynchronous transmitter release. *Neuron* 2000;26:683–694. [PubMed: 10896163]
- Massey PV, Bashir ZI. Long-term depression: multiple forms and implications for brain function. *Trends Neurosci* 2007;30:176–184. [PubMed: 17335914]
- Meir A, Ginsburg S, Butkevich A, Kachalsky SG, Kaiserman I, Ahdut R, Demigoren S, Rahamimoff R. Ion channels in presynaptic nerve terminals and control of transmitter release. *Physiol Rev* 1999;79:1019–1088. [PubMed: 10390521]
- Mendelowitz D, Reynolds PJ, Andresen MC. Heterogeneous functional expression of calcium channels at sensory and synaptic regions in nodose neurons. *J. Neurophysiol* 1995;73:872–875. [PubMed: 7760142]
- Mifflin SW. Short-term potentiation of carotid sinus nerve inputs to neurons in the nucleus of the solitary tract. *Respir. Physiol* 1997;110:229–236. [PubMed: 9407615]
- Mifflin SW, Felder RB. An intracellular study of time-dependent cardiovascular afferent interactions in nucleus tractus solitarius. *J. Neurophysiol* 1988;59:1798–1813. [PubMed: 3404205]
- Miles R. Frequency dependence of synaptic transmission in nucleus of the solitary tract in vitro. *J. Neurophysiol* 1986;55:1076–1090. [PubMed: 3012009]
- Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. *Physiol Rev* 1998;78:189–225. [PubMed: 9457173]
- Momiyama T, Koga E. Dopamine D(2)-like receptors selectively block N-type Ca(2+) channels to reduce GABA release onto rat striatal cholinergic interneurons. *J. Physiol* 2001;533:479–492. [PubMed: 11389206]
- Pongs O. Voltage-gated potassium channels: from hyperexcitability to excitement. *FEBS Lett* 1999;452:31–35. [PubMed: 10376673]
- Powell FL, Milsom WK, Mitchell GS. Time domains of the hypoxic ventilatory response. *Respir. Physiol* 1998;112:123–134. [PubMed: 9716296]
- Prabhakar NR, Kline DD. Ventilatory changes during intermittent hypoxia: importance of pattern and duration. *High Alt. Med. Biol* 2002;3:195–204. [PubMed: 12162863]
- Saha S, Spary EJ, Maqbool A, Asipu A, Corbett EK, Batten TF. Increased expression of AMPA receptor subunits in the nucleus of the solitary tract in the spontaneously hypertensive rat. *Brain Res. Mol. Brain Res* 2004;121:37–49. [PubMed: 14969735]
- Sara Y, Virmani T, Deak F, Liu X, Kavalali ET. An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. *Neuron* 2005;45:563–573. [PubMed: 15721242]
- Schild JH, Clark JW, Canavier CC, Kunze DL, Andresen MC. Afferent synaptic drive of rat medial nucleus tractus solitarius neurons: dynamic simulation of graded vesicular mobilization, release, and non-NMDA receptor kinetics. *J. Neurophysiol* 1995;74:1529–1548. [PubMed: 8989391]
- Sekizawa S, Bonham AC. Group I metabotropic glutamate receptors on second-order baroreceptor neurons are tonically activated and induce a Na⁺-Ca²⁺ exchange current. *J. Neurophysiol* 2006;95:882–892. [PubMed: 16192328]

- Sekizawa S, Joad JP, Bonham AC. Substance P presynaptically depresses the transmission of sensory input to bronchopulmonary neurons in the guinea pig nucleus tractus solitarius. *J. Physiol* 2003;552:547–559. [PubMed: 14561836]
- Seller H, Illert M. The localization of the first synapse in the carotid sinus baroreceptor reflex pathway and its alteration of the afferent input. *Pflugers Arch* 1969;306:1–19. [PubMed: 5813433]
- Shigetomi E, Kato F. Action potential-independent release of glutamate by Ca²⁺ entry through presynaptic P2X receptors elicits postsynaptic firing in the brainstem autonomic network. *J. Neurosci* 2004;24:3125–3135. [PubMed: 15044552]
- Talman WT, Perrone MH, Reis DJ. Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers. *Science* 1980;209:813–815. [PubMed: 6105709]
- Tang X, Dworkin BR. Baroreflexes of the rat. V. Tetanus-induced potentiation of ADN A-fiber responses at the NTS. *Am. J. Physiol Regul. Integr. Comp Physiol* 2007;293:R2254–R2259. [PubMed: 17913871]
- Tolstykh G, de Paula PM, Mifflin S. Voltage-dependent calcium currents are enhanced in nucleus of the solitary tract neurons isolated from renal wrap hypertensive rats. *Hypertension* 2007;49:1163–1169. [PubMed: 17372037]
- Turrigiano G. Homeostatic signaling: the positive side of negative feedback. *Curr. Opin. Neurobiol* 2007;17:318–324. [PubMed: 17451937]
- Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci* 2004;5:97–107. [PubMed: 14735113]
- Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat. Rev. Neurosci* 2007;8:935–947. [PubMed: 17987031]
- van Giersbergen PL, Palkovits M, De JW. Involvement of neurotransmitters in the nucleus tractus solitarius in cardiovascular regulation. *Physiol Rev* 1992;72:789–824. [PubMed: 1352638]
- Vidruk EH, Olson EB Jr, Ling L, Mitchell GS. Responses of single-unit carotid body chemoreceptors in adult rats. *J. Physiol* 2001;531:165–170. [PubMed: 11179400]
- Wierenga CJ, Walsh MF, Turrigiano GG. Temporal regulation of the expression locus of homeostatic plasticity. *J. Neurophysiol* 2006;96:2127–2133. [PubMed: 16760351]
- Xu J, Wu LG. The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron* 2005;46:633–645. [PubMed: 15944131]
- Zhang J, Mifflin SW. Integration of aortic nerve inputs in hypertensive rats. *Hypertension* 2000;35:430–436. [PubMed: 10642337]
- Zhang W, Mifflin SW. Modulation of synaptic transmission to second-order peripheral chemoreceptor neurons in caudal nucleus tractus solitarius by alpha1-adrenoreceptors. *J. Pharmacol. Exp. Ther* 2007;320:670–677. [PubMed: 17082311]
- Zhou Z, Champagnat J, Poon CS. Phasic and long-term depression in brainstem nucleus tractus solitarius neurons: differing roles of AMPA receptor desensitization. *J. Neurosci* 1997;17:5349–5356. [PubMed: 9204919]
- Zhou Z, Poon CS. Field potential analysis of synaptic transmission in spiking neurons in a sparse and irregular neuronal structure in vitro. *J. Neurosci. Methods* 2000;94:193–203. [PubMed: 10661839]