Information Processing in the Rostral Solitary Nucleus: Modulation and Modeling

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy
in the Graduate School of The Ohio State University

By
Alison Boxwell
Graduate Program in Neuroscience

The Ohio State University
2015

Dissertation Committee:
Dr. Joseph Travers, advisor
Dr. Susan Travers
Dr. David Terman
Dr. Candice Askwith
Abstract

The sense of taste contributes to quality of life and the vital function of discriminating between nutritive and toxic substances. It is intimately associated with appetitive and reward systems as well as homeostatic systems tasked with maintaining nutritional balance and body weight and is subject to widespread modulatory influences associated with digestive and satiety mechanisms including endogenous opioids. Central taste processing is initiated in the rostral nucleus of the solitary tract (rNST).

Although there is a significant literature describing fundamental biophysical properties of rNST neurons, their inputs from the solitary tract (ST), and inhibition arising from local interneurons, it is not known how these properties contribute to shaping the gustatory signal as it passes from peripheral afferents to central rNST neurons. In addition, some properties which have been demonstrated in the rNST (such as convergence of ST afferents, short term synaptic depression (STSD) of ST-evoked currents, and inhibition) may interact in ways which are difficult to address using conventional experimental methods. Intrinsic properties and their interactions are central to understanding how the rNST acts to shape the gustatory signal as a relay to higher central structures and to local medullary reflex circuits.

The work contained in this dissertation aims to determine the mechanism by which the activation of µ-opioid receptors modulates signal processing in the rNST, and finds that it is primarily a presynaptic inhibition of ST-evoked transmitter release. A second study combines in vitro recording and computational methods, measuring key features of both the ST-rNST synapse and postsynaptic rNST neurons in order to
generate a mathematical model of the system. This mathematical model is a tool for understanding the interactions between biophysical properties of the rNST network, exploring the effect of various forms of inhibition on gustatory signal processing, and generating testable predictions regarding modulation, convergence patterns of gustatory afferents, and rNST network configurations. In rNST neurons recorded in vitro, we find that the relationship between input frequency and total ST-evoked current is an increasing, saturating function and that the relationship between applied current and postsynaptic firing rate is characterized by a low threshold and a nearly linear relationship between applied current and firing frequency. When we fit a mathematical model to the in vitro data, we find that convergence and STSD interact to set an appropriate gain between ST input frequencies and rNST output frequencies, that convergence between ST afferents of the same best-stimulus type is required in order to recapitulate the response specificity observed in the rNST in vivo, and that presynaptic inhibition, postsynaptic inhibition, and inhibition by a broadly tuned inhibitory interneuron all function to improve the specificity of the modeled response to gustatory input.
Vita

2002-2005 .................................................. B.S. Neuroscience, The College of William and Mary

2005 to present  ........................................ MSTM / NGSP Student, The Ohio State University

Publications


Fields of Study

Major Field: Neuroscience
Table of Contents

Abstract............................................................................................................................... ii
Vita ........................................................................................................................................ iv
List of Tables..................................................................................................................... vi
List of Figures.................................................................................................................... vii
Chapter 1: Introduction....................................................................................................... 1
Chapter 2: The µ-Opioid Receptor Agonist DAMGO Presynaptically Suppresses Solitary
Tract-Evoked Input to Neurons in the Rostral Solitary Nucleus........................................... 17
Chapter 3: A Mathematical Model of Gustatory Processing in the Rostral Nucleus of the
Solitary tract ....................................................................................................................... 49
Chapter 4: Discussion......................................................................................................... 101
References .......................................................................................................................... 108

v
List of Tables

Table 1. Model Parameter Values

100
List of Figures

Figure 1.1 The rNST Circuit.................................................................16
Figure 2.1 EGFP Expression in the rNST.............................................38
Figure 2.2 Latency and Jitter...............................................................40
Figure 2.3 Recording Location.............................................................42
Figure 2.4 The Effect of DAMGO on ST-Evoked EPSCs..........................44
Figure 2.5 The Effect of DAMGO is Presynaptic.................................46
Figure 2.6 DAMGO Effects are Abolished by CTAP..............................48
Figure 3.1 Structure of the Mathematical Model..................................78
Figure 3.2 ST-Evoked Currents in rNST Neurons.................................79
Figure 3.3 Intrinsic Excitability of rNST Neurons................................81
Figure 3.4 Convergence in the rNST..................................................83
Figure 3.5 In Vivo Data.................................................................84
Figure 3.6 Baseline Model...............................................................86
Figure 3.7 The Effect of Stimulus-Specific Convergence on H and R........87
Figure 3.8 Presynaptic Inhibition.........................................................89
Figure 3.9 Postsynaptic Inhibition.........................................................91
Figure 3.10 Synaptic Inhibition..........................................................93
Figure 3.11 Synaptic Inhibition with a Broadly Tuned Surround.............95
Figure 3.12 Mean Response Profiles for In Vivo Data and Modeled rNST Responses........................................................97
Figure 3.13 Average Values for Frequency and Breadth of Tuning of Modeled rNST Neurons.................................................................99
Chapter 1: Introduction

The sense of taste contributes to the quality of life and the vital function of discriminating between nutritive and toxic substances. It is intimately associated with appetitive and reward systems as well as homeostatic systems tasked with maintaining nutritional balance and body weight, and is subject to widespread modulatory influences associated with digestive and satiety mechanisms (Berthoud, 2002; Grill & Hayes, 2012). Despite this central role in homeostatic and ingestive behavior, central nervous system mechanisms for processing and modulation of gustatory information are poorly defined relative to other sensory modalities. Dysregulation of appetitive and ingestive behaviors can result in obesity and its associated health consequences (Spielman, 1998), and understanding the mechanisms which underlie transduction and modulation in the gustatory system is an important component of understanding how this sensory system contributes to health and disease.

Overview of the Gustatory System

The rostral nucleus of the solitary tract (rNST) contains the first central synapse in the afferent taste pathway (see Figure 1.1), and functions to collect taste information from the periphery and distribute it to ascending and local reflex pathways (Lundy and Norgren, 2004) Gustatory information and mechanosensory information are carried by the 7th, 9th, 10th, and 5th (purely mechanosensory) cranial nerves, which synapse centrally in the rostral nucleus of the solitary tract (rNST). Neurons in the rNST receiving gustatory input in turn project to higher brain structures (primarily the
parabrachial nucleus) or local medullary structures (primarily the subjacent reticular formation and caudal NST) (Halsell, Travers, & Travers, 1996). The ascending pathway is important for taste perception, the hedonic aspects of gustatory function, and some aspects of homeostasis, while the local reflexive/visceral pathway is important for reflexive oromotor behaviors (consummatory responses of ingestion and rejection in response to appetitive and aversive stimuli) and integration of taste and visceral function, e.g. satiety (reviewed in (Spector & Travers, 2005).) Thus, processing of a sensory signal at the level of the rNST has the potential to influence the diverse functions of both the ascending and local reflex pathways, and may therefore influence a wide range of behaviors with significant implications for health and disease.

The function of the gustatory system is to identify the nature and concentration of taste stimuli in the oral cavity. In contrast to the stimulus dimension of other sensory systems (e.g. electromagnetic wave length, sound pressure), taste stimuli do not fall along a single easily defined continuum, but instead fall into one of five stimulus classes that are perceived as sweet, salty, acidic, bitter, or umami. Stimuli evoking these perceptions do not fall into discrete physico-chemical classes, e.g. the heavy metal lead and many carbohydrates both taste “sweet”. Although the ion channels and G-protein coupled receptors responsible for signal transduction in the tongue are segregated within individual cells in the taste bud (Chaudhari & Roper, 2010), some taste bud cells and the peripheral afferents which carry taste information to the central nervous system are often responsive to diverse stimuli (Barretto et al., 2014; Frank, Contreras, & Hettinger, 1983; Tomchik, Berg, Kim, Chaudhari, & Roper, 2007; Yoshida et al., 2009).

*The Fundamental Coding Problem in Taste*

The sensitivity of gustatory afferents and rNST neurons to multiple stimuli frames the basic question of how taste is encoded in the brain (Woolston & Erickson, 1979). The theory that taste quality and intensity are encoded in the firing frequency to the most
effective “best” stimulus of individual neurons is termed a “labeled line” code. Since firing frequency generally increases with stimulus concentration in both gustatory afferents (Frank et al., 1983; Ogawa, Sato, & Yamashita, 1968; Pfaffmann, 1941) and rNST neurons (Ganchrow & Erickson, 1970; S. P. Travers, Pfaffmann, & Norgren, 1986; Victor & Di Lorenzo, 2011), a particular firing frequency in an individual neuron may result from a low concentration of an effective stimulus or a high concentration of a less effective stimulus. If the brain encodes taste using a labeled line code, this ambiguity results in taste quality being confounded with concentration—such as, for example, a high concentration of salt might activate a pathway that signals “sweet”. This problematic ambiguity is resolved by the theory that taste is encoded in the activity of an ensemble of neurons (an “across-fiber” code), and both the identity and intensity of a stimulus may be extracted by comparing activity of many individual neurons with different sensitivities to taste stimuli (Erickson, Doetsch, & Marshall, 1965).

The sensitivity of gustatory afferents and neurons to multiple stimuli also poses the methodological question of how to compare gustatory responses across experimental conditions, anatomical locations, and between species. It is possible to classify units according to their best-stimulus and compare the magnitude of the change in firing frequency, but this only captures one aspect of the response. In order to draw useful comparisons, an additional metric is required which captures the overall profile of each unit’s response, from a unit which responds equally to all tested stimuli to one which only responds to one. The “breadth of tuning” refers to the selectivity of a unit’s response; an equal response to all taste stimuli is a very broadly tuned response, while a response to only one is very narrowly tuned. Two commonly used metrics of breadth of tuning (BOT) are entropy (J. B. Travers & Smith, 1979) and the noise/signal ratio (Spector & Travers, 2005). Entropy has roots in information theory and is calculated using all measured responses, while the noise/signal ratio is the ratio of the number of spikes to the second most effective stimulus divided by the number of spikes to the most effective. Both metrics range from 0 (narrowly tuned) to 1 (broadly tuned). The advantages and...
disadvantages of each metric are discussed in (Spector & Travers, 2005). Briefly, entropy better reflects the overall response profile because it takes into account all of the responses, however, the formula for entropy does not handle negative numbers, i.e. inhibitory responses. On the other hand, the noise to signal ratio rarely confronts this problem because the response to the 2nd best stimulus is virtually never inhibitory, but this measure ignores the 3rd and 4th-best stimulus responses.

When entropy was first introduced as a measure of the breadth of tuning, it was used to show that the gustatory responses of the rNST were more broadly tuned than their chorda tympani (CT) inputs (J. B. Travers & Smith, 1979). Although there is considerable variability in both firing frequency and breadth of tuning within populations of peripheral afferents and central rNST neurons, there is a clear tendency for taste responses to become both faster and more broadly tuned in the rNST (see Spector & Travers, 2005 for a meta-analysis).

The rNST functions to both relay and process the gustatory signal, and the relationship between input to the rNST and its output defines a “transfer function”. The increases in firing frequency and BOT observed between gustatory afferents and rNST neurons reflect the processing of the gustatory signal in the rNST and are the result of the shape of the rNST transfer function. The transfer functions of individual neurons may vary considerably around this mean behavior, and there may also be systematic differences in the behavior of rNST neurons participating in different pathways (e.g. pathways which contribute to discriminative vs. reflexive behavior) or those serving different functions within the same pathway (e.g. excitatory vs. inhibitory; projection neuron vs. interneuron).

The transfer function of an individual rNST neuron is a consequence of its cellular properties and surrounding network organization, including the expression of specific ion channels, short term synaptic plasticity at the afferent synapse, and network
properties like convergence and inhibition. These will be discussed in more detail below. Even when these mechanisms are well-studied, it has proven to be very difficult to connect mechanistic data (typically measured in vitro) to measurements of function such as firing frequency and breadth of tuning (typically measured in vivo), and this connection is important for understanding both how rNST neurons process gustatory information and how this processing may be modulated in health and disease. In Chapter 3, we use a mathematical model based on the known cellular and network properties of the rNST as a tool to bridge this gap. In addition, we consider how various modulatory mechanisms are likely to influence the rNST transfer function, with predicted consequences for both the firing frequency and BOT.

**Neuron Types in the rNST**

rNST neurons may be characterized as either interneurons or projection neurons, and projection neurons participate in either an ascending pathway via the parabrachial nucleus or a local medullary reflex pathway via the subjacent reticular formation (see Figure 1.1) (Halsell et al., 1996). PBN projection neurons are enriched in the rostrocaudal subdivision of the rNST, while RF projection neurons are enriched in the ventral subdivision (Halsell et al., 1996). There is some evidence that these pathways are segregated as early in the gustatory pathway as the primary afferents (Zaidi, Todd, Enquist, & Whitehead, 2008).

The presence of these parallel circuits in the rNST is important to note since neurons participating in each pathway may have different properties suited to their functional roles, including different afferent input characteristics (M. Wang & Bradley, 2010b) and ion channel expression patterns (Corson & Bradley, 2013; Suwabe & Bradley, 2009). Much more is known about the biophysical properties of rNST neurons and their cranial nerve inputs as a whole than is known about the differences between the two functional pathways.
Most rNST neurons receive monosynaptic glutamatergic (primarily AMPA receptor dependent but with some contribution from NMDA receptor activation) input from the solitary tract (L. Wang & Bradley, 1995). The solitary tract is made up of the chorda tympani, glossopharyngeal, and vagus nerves, which have overlapping but ordered terminal fields in the rNST (Hamilton & Norgren, 1984). This gives rise to a significant degree of convergence between these nerves as well as to a loosely organized orotopic map within the rNST (S. P. Travers & Norgren, 1995). Many neurons receive input from both the chorda tympani and glossopharyngeal nerves, and the postsynaptic responses to these inputs usually sum independently (Grabauskas & Bradley, 1996).

Convergence of afferents increases both the size of receptive fields and the reliability of stimulus detection, and it has long been hypothesized that it is also responsible for the increase in firing frequency and breadth of tuning in the rNST compared to its CT inputs (J. B. Travers & Smith, 1979). That this convergence occurs has been demonstrated in vivo by studies which show that rNST receptive fields are larger than those of peripheral afferents (Vogt & Mistretta, 1990) and that these receptive fields may include different anatomical locations within the oral cavity which may have qualitatively different response characteristics, suggesting that their responses are mediated by multiple afferents with different best-stimuli (Sweazey & Smith, 1987; S. P. Travers et al., 1986).

Although convergence, particularly between afferents with different gustatory responsiveness, may explain the observed increase in response frequency and BOT at the level of the rNST, it is unlikely to be the only factor involved in this transformation. The average number and pattern of convergent afferents in the rNST is unknown, as is the effect of these variables on the firing frequency and BOT of rNST neurons. By creating a
mathematical model of the rNST, it is possible to work backwards from the observed change in the firing frequency and BOT to make an educated hypothesis about what numbers and patterns of convergent inputs are likely to exist in the rNST. Experimental testing of this hypothesis would be difficult but not impossible using existing techniques, and an understanding of how convergence affects gustatory processing may reveal important questions for further investigation.

*rNST Processing Mechanisms: Short Term Synaptic Depression*

Short term synaptic depression (STSD) is feature of ST afferents synapsing in the rNST which could also significantly contribute to an increase in breadth of tuning in central taste responses (M. Wang & Bradley, 2010b). STSD is a phenomenon which results in a smaller response to afferent stimulation when the same afferent has been stimulated very recently. The time scale of this effect is typically on the order of less than one second, with more pronounced effects at much shorter interstimulus intervals (Zucker & Regehr, 2002). When multiple electrical stimuli are applied to gustatory nerves in vivo, STSD manifests as an increased failure rate to the second stimulus with short interstimulus intervals (Hallock & Di Lorenzo, 2006; Lemon & Di Lorenzo, 2002; Rosen & Di Lorenzo, 2009). Although it had been hypothesized that this phenomenon could be the result of the recruitment of recurrent inhibition, it persists even when inhibition is blocked in in vitro preparations where it manifests as a reduction in the amplitude of ST-evoked EPSCs with increasing frequency and duration of tract stimulation (M. Wang & Bradley, 2010b).

Short term synaptic depression has been described as a mechanism for frequency-dependent filtering (Fortune & Rose, 2001), and it could potentially result in an increase in the breadth of tuning in the rNST by faithfully passing low-frequency inputs while filtering the passage of high-frequency inputs, thereby amplifying the sideband sensitivities relative to the best-stimulus response. In other systems, STSD has also been
described as a mechanism for gain-setting (a proportional scaling of the response to 
input) (Abbott, Varela, Sen, & Nelson, 1997) and as a mechanism for accentuating 
changes in input firing rate (MacLeod, 2011), however the exact nature of its function in 
the rNST has not been determined.

*rNST Processing Mechanisms: Intrinsic Excitability and Constituent Ion Channels*

The intrinsic excitability of rNST neurons has been extensively characterized by 
the application of one or a few levels of positive injected currents, typically 0.1 nA, to 
which they respond with modest firing rates (Bradley & Sweazey, 1992; Du & Bradley, 
1996; Suwabe & Bradley, 2009; Suwabe, Mistretta, Krull, & Bradley, 2011; Tell & 
Bradley, 1994; M. Wang & Bradley, 2010a). Some effort has been expended to 
categorize these neurons into types or groups on the basis of their responses to these 
applied currents following a brief period of hyperpolarization, and two notable features of 
rNST neuron responses have been consistently observed: the presence of a “sag” in the 
current during the hyperpolarization step consistent with the presence of the 
hyperpolarization-activated mixed cationic channel IH and/or the presence of a 
significant delay in rNST neuron firing following the hyperpolarizing step consistent with 
the presence of the hyperpolarization-deinactivated potassium current IA (Bradley & 
Sweazey, 1992). These currents have been characterized in voltage clamp (Tell & 
Bradley, 1994), and it has been shown that these characteristics as well as the expression 
of these currents change over the course of development (Suwabe et al., 2011).

Although some studies have found that IA and IH are expressed in separate 
subsets of rNST neurons (Uteshev & Smith, 2006; M. Wang & Bradley, 2010a), others 
have demonstrated significant coexpression (Suwabe et al., 2011). One study 
demonstrating that inhibitory rNST neurons frequently express IH but never IA (M. 
Wang & Bradley, 2010a) suggests that there may be systematic differences between the 
excitability and sensitivity to inhibition of excitatory and inhibitory rNST neurons. This
study was performed in current clamp and may have missed IA currents which are small, have a short time course, or have a voltage dependence which results in partial deinactivation at the artificially induced resting membrane potential of -60 mV, but it is an interesting experiment that would be worth repeating using more sensitive voltage clamp protocols.

Although the idea that IA and IH may be differentially expressed in excitatory and inhibitory rNST neurons is intriguing, a more basic question is whether these neurons have significantly different postsynaptic properties or input characteristics which might result in differences in the processing of gustatory information. Experiments presented in Chapter 3 address this question by measuring characteristics of ST afferents and intrinsic excitability of rNST neurons in a mouse model expressing the yellow fluorescent protein VENUS in glycinergic and GABAergic neurons.

*rNST Processing Mechanisms: Inhibition*

In addition to the monosynaptic input from the ST to rNST neurons, there is a polysynaptic inhibitory pathway in the rNST (see Figure 1.1), as evidenced by GABAA-mediated IPSCs (L. Wang & Bradley, 1995) in many rNST neurons in response to ST stimulation. When local inhibition is evoked by direct stimulation of the rNST in the presence of glutamate blockers, it is characterized by slow decay kinetics, limited synaptic depression, temporal summation, and sustained activation during and following tetanic stimulation (Grabauskas & Bradley, 1999; 2003). GABAergic terminals have been shown to synapse onto both gustatory neurons and ST terminals (Leonard, Renehan, & Schweitzer, 1999; Whitehead, 1986), providing anatomical indication that inhibition may also act to suppress ST-evoked input to rNST neurons via a presynaptic pathway, consistent with a similar mechanism described in the caudal NST (Fawley, Peters, & Andresen, 2011).
Tonic GABAergic inhibition has been proposed as a mechanism to sharpen the breadth of tuning of rNST neurons. In vivo microinjection of GABA suppresses the spontaneous and taste-evoked responses of rNST neurons (Smith & Li, 2000), and microinjection of the GABAA antagonist bicuculine releases tonic inhibition in gustatory neurons, resulting in a roughly equivalent increase in the response to multiple tastants (Smith & Li, 1998). This increases the breadth of tuning in these neurons, suggesting that tonic inhibition normally functions to sharpen the breadth of tuning in the rNST. Since tonic inhibition has been demonstrated in an in vitro slice preparation (Smith & Li, 1998), this tonic inhibition is likely at least partially mediated by tonically active medullary interneurons, although it could also be a function of taste-responsive inhibitory rNST neurons or the result of a modulatory influence from other brain regions such as the insular cortex (Smith & Li, 2000).

Just as convergence and STSD have been discussed as mechanisms which may increase the BOT in the rNST, tonic inhibition has been discussed as a mechanism for sharpening the BOT (Smith & Li, 1998). The model presented in Chapter 3 attempts to quantify the relative and opposing contributions of these various mechanisms, including several different modulatory mechanisms with qualitatively different effects on the BOT of modeled rNST taste responses.

Modulation

Modulation of gustatory responses in the rNST has the potential to impact a wide range of discriminative, hedonic, homeostatic, and reflexive behaviors, and so the mechanisms of normal regulation are important to understand. If normal signal processing in the rNST can be thought of in terms of a relationship between an input and an output, modulation can be thought of in terms of an alteration in that relationship. This alteration is often state-dependent (e.g. hungry vs. sated) and may be the result of both humoral and neural factors (Scott & Verhagen, 2000). The activity of the rNST has
been shown to be modulated by gastric distention (Glenn & Erickson, 1976), as well as the levels of glucose, insulin, and glucagon in the blood (Giza, Scott, & Vanderweele, 1992), consistent with the role of these factors in modulating taste and feeding behavior. The rNST receives projections from a number of centrifugal sources that utilize GABA, substance P, endogenous opioids, neuropeptide Y, norepinepherine, nitric oxide, and dopamine which may mediate these effects. In addition, somatostatin and receptors for serotonin and histamine have been observed in the rNST and may play a modulatory role (reviewed in Lundy and Norgren, 2004). The response properties of rNST neurons may also be altered by learning, as when a novel flavor is paired with an aversive stimulus (Chang & Scott, 1984). Although modulation of rNST activity is both common and important, the cellular mechanisms of modulatory systems in the rNST are often incompletely understood.

The work contained in Chapter 2 of this dissertation aims to elucidate the mechanisms by which the activation of µ-opioid receptors modulate signal processing in the rNST. Opioid receptor expression has been demonstrated in the rNST (Li et al. 2003), and the mixed µ / δ ligand met-enkephalin has been shown to profoundly suppress rNST gustatory responses in vivo (Li et al. 2003). Previous work in our lab has shown that the infusion of the µ-opioid agonist DAMGO into the rNST alters oromotor reflex behaviors which are a product of local medullary reflex pathways in the medulla (Kinzeler & Travers, 2011). Despite the demonstrated modulatory effects of opioids in the rNST, their location and mechanism of action have not been well studied. Activation of δ-opioid receptors has been shown to postsynaptically suppress ST-evoked responses in the rNST (Zhu et al. 2009), and the work presented in Chapter 2 demonstrates that activation of µ-opioid receptors presynaptically suppresses ST-evoked responses in the rNST.

Predicting the functional consequences of this modulation depends on understanding how the intrinsic properties of the rNST affect the transfer function from
solitary tract afferents to rNST projection neurons. Although there is a significant literature describing fundamental biophysical properties of rNST neurons, their synaptic inputs from the solitary tract (ST) and inhibition arising from local interneurons, this work is insufficient to allow a confident prediction of how presynaptic inhibition by DAMGO might alter the transduction of a natural gustatory signal through the rNST.

Specifically, it is difficult to predict how a nearly 50% presynaptic reduction in ST-evoked EPSCs influences postsynaptic firing frequency or BOT in rNST neurons. It may be tempting to posit that the firing frequency to any given gustatory stimulus is also reduced by 50%, and the rNST neurons’ BOT is therefore unaffected (since both common metrics of BOT are based on proportional measurements). However, this is certainly an oversimplification that does not take into account important features of the system, in particular the tendency for presynaptic inhibition to interact significantly with afferents’ STSD over the course of a prolonged input.

One of the main mechanisms underlying STSD is often the depletion of the readily releasable pool of vesicles (Zucker & Regehr, 2002), and this depletion may be reduced when release is suppressed. As a result, presynaptic inhibition can contribute to a relative increase in readily releasable vesicles (which tends to increase the size of subsequent EPSCs), in opposition to its direct effect (which tends to decrease the size of all EPSCs). As these opposing effects come into equilibrium, the net effect is that presynaptic inhibition has a significantly larger impact on the first response to afferent stimulation (where the magnitude of presynaptic inhibition is typically quantified) than on later responses in a train of stimuli. The consequent temporal pattern in the magnitude of presynaptic inhibition complicates an analysis of its effect on a system where the natural stimuli may last several seconds.

This illustrates the fundamental difficulty of connecting mechanistic results from in vitro studies with functional results from in vivo studies, and this problem is by nature
difficult to approach using conventional experimental techniques. For example, it is unclear how intrinsic properties measured in vitro, such as the resting membrane potential or the size of the ST-evoked EPSC, contribute to the transformation in firing frequency and breadth of tuning measured in peripheral afferents and central rNST neurons in vivo. Consequently, it is difficult to predict how alteration of these intrinsic properties (which may be changed by the application of a drug, over the course of development, or in a disease state) may affect processing in the rNST.

Mathematical modeling

One approach to understanding how the intrinsic properties of the rNST circuit (as measured in vitro) contribute to shaping the gustatory signal (as measured in vivo) is mathematical modeling. The work presented in Chapter 3 combines in vitro studies and computational methods to generate a biologically informed mathematical model of the rNST. We use the model to address a number of long-standing hypotheses in the gustatory system, including (1) the hypothesis that convergence increases the breadth of tuning in the rNST compared to the CT, (2) that short term synaptic depression (STSD) produces a low-pass filter between gustatory afferents and rNST neurons, and (3) that inhibition sharpens the breadth of tuning in the gustatory rNST. We also address several new questions regarding the functional consequences of various mechanisms of modulation in the rNST.

Our model is consistent with what is known about the gustatory rNST from both in vivo and in vitro studies. In order to be able to relate the performance of the model to rNST function, we used in vivo CT data (Frank, 1973) as input and compared the modeled output to corresponding in vivo data from the rNST (J. B. Travers & Smith, 1979). This approach allows us to predict the effect of modulatory mechanisms on the firing frequency and BOT of rNST neurons in response to taste stimuli.
The core of the model is a Hodgkin-Huxley type conductance based model of a rNST neuron, tuned so that the function between input current and output firing frequency resembles the average function measured in both excitatory and inhibitory rNST neurons in vitro. This neuron model receives modeled solitary tract input in the form of a synaptic conductance which closely matches in vitro recordings. Specifically, a 3-compartment model of transmitter release (Ermentrout and Terman, 2010) was fit to the observed relationship between input frequency and synaptic response magnitude, which is influenced by both the amplitude of individual EPSCs and the frequency dependence and degree of STSD.

We modeled up to 10 individual afferents in this way, and in order to ensure that there were no artifacts of input synchronicity we used the input firing frequency to generate a unique pattern of poisson-distributed spike times for each afferent. These spike times drive the model of transmitter release, which drive an output firing frequency in the modeled rNST neurons. We use a python script to randomly select CT data to use as input firing frequencies and collect the corresponding modeled rNST responses. In this way, we are able to generate a modeled response to the application of four different gustatory stimuli, and compare the firing frequency and BOT of this modeled response to in vivo rNST data (J. B. Travers & Smith, 1979).

In terms of complexity, our model occupies a middle ground. There are certainly more complex synaptic and neuronal models, such as the gorgeously detailed and mechanistic effort to model synaptic release (Schild, Clark, Canavier, Kunze, & Andresen, 1995) and neuronal excitability (Schild et al., 1993) in the caudal NST. These models are excellent for generating testable predictions regarding very specific mechanisms, and the contributions of these mechanisms to phenomena observed in vitro (like STSD). There are also models which are significantly less complex, such as models of STSD which calculate EPSC amplitude as a function of the time since the last event or integrate and fire neuronal models (discussed in Ermentrout and Terman, 2010). These models are
excellent for scaling up into network simulations, but may fail to capture some important features of rNST responses. The form we chose for our model allows us to scale it up to a very limited network (2 neurons with up to 10 afferents) while maintaining a degree of mechanistic faithfulness which allows us to capture features of the in vitro behavior which we believe are relevant to determining the transfer function in these neurons. By occupying this middle ground, we hope to be able to bridge the gap between mechanism and function in the rNST.
Figure 1.1
The rNST Circuit

A circuit diagram of the rostral nucleus of the solitary tract, showing afferent input (orange), projection neurons (red), inhibitory interneurons (blue), and descending projections (black). (PBN= parabrachial nucleus; RC= rostrocentral subdivision; V= ventral subdivision; RL= rostrolateral subdivision; M= medial subdivision). Adapted from Travers & Travers, 2010.
Chapter 2: The µ-Opioid Receptor Agonist DAMGO Presynaptically Suppresses Solitary Tract-Evoked Input to Neurons in the Rostral Solitary Nucleus

Abstract

Taste processing in the rostral nucleus of the solitary tract (rNST) is subject to modulatory influences including opioid peptides. Behavioral pharmacological studies suggest an influence of µ-opioid receptors in rNST, but the underlying mechanism is unknown. To determine the cellular site of action, we tested the effects of the µ-opioid receptor agonist DAMGO in vitro. Whole cell patch clamp recordings were made in brainstem slices from GAD67-GFP knock-in mice expressing EGFP under the control of the endogenous promoter for GAD67, a synthetic enzyme for GABA. Neuron counts showed that ~36% of rNST neurons express GABA. We recorded monosynaptic solitary tract (ST)-evoked currents (jitter < 300 µs) in both GAD67-EGFP positive (GAD67+) and GAD67-EGFP negative (GAD67-) neurons with equal frequency (25/31;22/28), but the inputs to the GAD67+ neurons had significantly smaller paired pulse ratios compared to GAD67- neurons. DAMGO (0.3 µM) significantly suppressed ST-evoked currents in both cell types (mean suppression = 46+/− 3.3 % SEM), significantly increased the paired-pulse ratio of these currents, and reduced the frequency of spontaneous miniature EPSCs but did not diminish their amplitude, indicating a presynaptic site of action. Under inhibitory amino acid receptor blockade, DAMGO was significantly more suppressive in GAD67+ neurons (59% reduction) compared to GAD67- neurons (35% reduction), while the reverse was true in normal ACSF (GAD67+: 35% reduction;
GAD67:- 57% reduction). These findings suggest that DAMGO suppresses activity in rNST neurons predominantly via a presynaptic mechanism, and that this effect may interact significantly with tonic or evoked inhibitory activity.

Introduction

The rostral nucleus of the solitary tract (rNST) is the site of the first central synapse in the afferent taste pathway, receiving input from the 7th, 9th, and 10th cranial nerves (Lundy and Norgren 2004). Although it serves as a distribution node for both local and ascending gustatory pathways, it also constitutes a complex intrinsic processing network subject to modulatory influences from forebrain gustatory and homeostatic regions (Bradley 2007; Lundy and Norgren 2004; Smith and Travers 2008). Opioids are one among several classes of neuromodulators that have been demonstrated to be present and/or affect taste responses in the rNST (Davis and Smith 1997; King et al. 1993; Li et al. 2003; Travers and Travers 2010) and opioid receptors, including the μ and δ subtypes are located in the rostral, gustatory as well as caudal visceral regions of the nucleus (Huang et al. 2000; Li et al. 2003; Mansour et al. 1994; Monteillet-Agius et al. 1998; Moriwaki et al. 1996). Indeed, depending on the precise location, intra-NST infusions of opioids affect not only gustatory, but also visceral and ingestive functions (Herman et al.; Kinzeler and Travers 2011; Kotz et al. 1997).

In the caudal nucleus of the solitary tract (cNST) and associated dorsal motor nucleus of the vagus, μ-opioid receptors (MORs) act at multiple loci, exerting not only presynaptic inhibitory effects on primary afferent fibers, but also postsynaptic inhibitory effects on intrinsic neurons (Appleyard et al. 2005; Poole et al. 2007; Rhim et al. 1993; Rhim and Miller 1994). In addition, MOR activation in this region has a powerful effect on vago-vagal reflexes controlling gastric function and tone. Interestingly, a recent study (Herman et al. 2012) demonstrated gastric effects due to interactions between the manipulation of cNST μ-opioid and GABAergic receptors consistent with effects on cNST neurons observed in vitro (Herman et al. 2010).
In the rNST, microinjection of the mixed \( \mu / \delta \) ligand, met-enkephalin, suppresses taste-evoked responses (Li et al. 2003) and \textit{in vitro} studies suggest that an underlying basis for this effect is \( \delta \)-opioid receptor-mediated postsynaptic inhibition of (excitatory) parabrachial nucleus (PBN)-projection neurons (Zhu et al. 2009). However, immunohistochemical studies imply that MORs are also located on primary gustatory afferents (Li et al. 2003). Moreover, infusion of the MOR agonist, DAMGO, into this substrate results in complex changes in oromotor reflex behaviors that include abbreviating the duration of lick bouts and augmenting the oral rejection response, effects compatible with underlying inhibitory and disinhibitory processes, respectively (Kinzeler and Travers 2011). Thus, it would appear that in addition to postsynaptic influences of \( \delta \)-receptors on excitatory projection neurons, opioid mechanisms in the rNST include \( \mu \)-opioid receptor mediated effects exerted through other mechanisms or cell types, similar to their effects in the caudal region of the nucleus.

To begin to explore the cellular basis of \( \mu \)-opioid influence in rNST, we examined the effect of DAMGO on solitary-tract (ST)-evoked responses in an \textit{in vitro} slice preparation in a strain of transgenic mice that express EGFP under the control of the endogenous promoter for GAD67, a synthetic enzyme for GABA (Tamamaki et al. 2003). We determined that DAMGO had a primarily presynaptic suppressive effect on ST-evoked input to both GAD67-EGFP positive (GAD67+) and GAD67-EGFP negative (GAD67-) rNST neurons. Recording from this model also enabled us to characterize synaptic properties of these identified neurons, and we found that monosynaptic input to GAD67+ neurons was characterized by a significantly smaller paired-pulse ratio than was input to GAD67- neurons.

\textit{Methods}

\textit{Animal Model}

We used a transgenic mouse expressing EGFP under the control of the endogenous promoter for GAD67, a synthetic enzyme for GABA. In contrast to the
commercially available “GIN” mouse, which only expresses GFP in a particular subset of rNST GABAergic neurons (Travers et al. 2007; Wang and Bradley 2010a), this GAD67-GFP knock-in strain has been shown to produce EGFP in virtually all cells expressing GAD67 (Brown et al. 2008; Kaneko et al. 2008; Ono et al. 2005; Tamamaki et al. 2003), allowing for a more complete identification of GABAergic neurons in the NTS. Because of reports that monosynaptic inputs to GABAergic neurons in the rNST may diminish during development in rat (Wang et al. 2012), but evidence for pre-synaptic inhibition of monosynaptic vagal inputs to cNST GABAergic neurons in mature as well as young mice (Glatzer et al. 2007), we included both mouse pups (P14-18) and adult mice (6 – 11 weeks) in our study. Initially we tested mouse pup brain slices in ACSF and sometimes observed both inhibitory and excitatory inputs following afferent stimulation that precluded measurement of jitter essential to an interpretation of monosynaptic input. We therefore recorded from a population of rNST neurons while suppressing inhibitory currents with a bath application of a gabazine/strychnine cocktail. This latter protocol was maintained in the adult group. All experimental protocols were approved by the Ohio State University Institutional Animal Care and Use Committee in accordance with guidelines from the National Institutes of Health.

Slice Preparation

Acute slices were prepared for electrophysiological recording following anesthetization with urethane (33% urethane, 10mL/kg), decapitation, and rapid removal and cooling of the brain. In in vitro experiments, urethane has been shown to wash out in considerably less time than our hour long incubation step prior to recording and thus it was unlikely to have had any influence on our neurons at the time of recording (Hara and Harris 2002; Sceniak and Maciver 2006). The blocked brainstem was glued to a ceramic block using cyanoacrylate glue, and coronal brainstem slices 250-300 μm thick were cut with a sapphire blade on a vibratome (model 1000, Vibratome, St. Lois, MO, USA) in an ice-cold carboxygenated cutting solution containing (in mM) 110 choline, 25 NaHCO3, 3 KCl, 7 MgSO4, 1.5 NaH2PO4, 10 d-Glucose, 0.5 CaCl2. Slices containing the NST
rostral to where it moves laterally away from the fourth ventricle were identified and incubated in a carboxgyenated artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 25 NaHCO3, 3 KCl, 1 MgSO4, 1.5 NaH2PO4, 10 d-Glucose, and 1.5 CaCl2 at 36°C for one hour prior to recording.

_Electrophysiological Recording and Stimulation of the Solitary Tract_

Slices were transferred to a recording chamber and perfused with 36°C ACSF at a rate of 1-2 mL/minute. The NST and solitary tract (ST) were visualized with a Nikon E600FN microscope and a bipolar stimulating electrode made of twisted insulated wire was placed on the ST under visual control. Stimulation of the ST consisted of two current pulses 0.15 ms in duration separated by 33 ms, and delivered at 0.3 hertz. The interstimulus interval (ISI) of 33 ms was chosen to minimize summation between the responses to the first and second stimuli while also allowing the first stimulus to influence the probability of release in response to the second stimulus. It also falls within the range of ISI’s used by other investigators studying this brain region (Appleyard et al. 2005; Zhu et al. 2009). Threshold was defined as the current intensity that evoked a response 50% of the time, and the experimental stimulus intensity was set at 20 µA above the threshold to ensure a reliable response. In some cases, the presence of an additional, higher-threshold evoked response precluded the use of current values 20 µA above threshold, and in these cases the highest current that did not activate the additional input was used. Stimulus intensities ranged from 5 to 140 µA.

After placement of the stimulating electrode, cells in the rNST were visualized using IR-DIC optics and epifluorescent illumination to identify cells which were either GAD67+ or GAD67-. Approximately equal numbers of GAD67+ and GAD67- neurons were targeted throughout the course of the study. Once a neuron was identified, a whole cell patch clamp recording was made using a 4-6 MΩ pulled glass pipette filled with an intracellular solution containing (in mM) 130 K-gluconate, 10 EGTA, 10 HEPES, 1 CaCl2, 1 MgCl2, and 2 ATP, at pH 7.2-7.3 and osmolality 290-295 mOsm. An initial seal of greater than 1 GΩ and a membrane resistance of greater than 100 mΩ were
inclusion criteria for seal and cell viability. Recordings were made with an A-M systems Model 2400 amplifier and recorded using pClamp software (Molecular Devices, Sunnyvale, CA).

Recording Protocols

Cells were initially held in current clamp and injected with 1 second current steps sufficient to elicit action potentials (typically 0.05-0.1nA). Their response to ST stimulation was assessed in voltage clamp at -70 mV to record evoked excitatory postsynaptic currents (EPSCs) and in a few cases at -40 mV to record evoked (inhibitory) IPSCs. Cells which did not display a positive action potential overshoot or a repeatable, time-locked response to ST stimulation were excluded from further study; such exclusions were very rare. In a separate group of cells, we measured the effect of DAMGO on miniature EPSCs in the presence of 1 µM tetrodotoxin (TTX), 2 mM CaCl2, 5 µM gabazine, and 5 µM strychnine. These cells were held in voltage clamp at -70 mV and were included in the study if their baseline rate of miniature EPSCs exceeded 0.5 Hz.

Drug Conditions, Tracers, and Immunohistochemistry

DAMGO was bath applied to each cell at 0.3 µM for 5.5 minutes, followed by a washout period in normal ACSF. This concentration fell in the middle of the range of concentrations reported in the literature (Herman et al.; Poole et al. 2007; Rhim et al. 1993; Rhim and Miller 1994) and was ideal for our purposes because in most cases it caused considerable but incomplete suppression of the evoked response, which allowed us to measure a paired pulse ratio (PPR) throughout the protocol. One of 3 baseline drug conditions was used: either normal ACSF as described above, normal ACSF containing 5 µM gabazine and 5 µM strychnine, or normal ACSF containing gabazine, strychnine, and 1 µM CTAP, a MOR antagonist (Glatzer et al. 2007; Glatzer and Smith 2005). Gabazine, strychnine, and CTAP, when used, were applied to the cell for a minimum of 15 minutes prior to the collection of baseline data, and were maintained throughout application of DAMGO and washout.
Four groups of data were collected using (1) mouse pups in ACSF (n=16 neurons), (2) mouse pups in gabazine/strychnine (n=23 neurons), (3) adult mice in gabazine/strychnine (n=12 neurons), and (4) adult mice in gabazine/strychnine/CTAP (n=5 neurons). In addition, TTX-resistant miniature EPSCs were recorded in 7 rNST neurons in order to provide additional information about the mechanism (pre- or postsynaptic) of DAMGO suppression.

In most instances, the location of the electrode was determined from images taken at the time of recording. In a few cases (n=4), cells were filled with 0.5% biocytin. After recording, slices were fixed in 4% paraformaldehyde and processed with a strepavidin-conjugated AlexaFluor to visualize the biocytin-filled neuron and processes and immunostained for the P2X2 receptor (P2X2r), a putative marker for gustatory afferents (Finger et al. 2005) in the rostral part of the nucleus. Briefly, slices were fixed for 24-48 hours, followed by rinses in phosphate buffered saline (PBS), blocking in 5% donkey serum in PBS/0.3% Triton X-100 for one hour, and incubated with the primary rabbit anti-P2X2 antibody (Alomone Labs, 1:10000) at 4°C for 48 hours. Following PBS rinses, the tissue was incubated overnight at 4°C with the secondary antibody (fluorescent donkey anti-rabbit IgG; 1:500 dilution, Invitrogen), then rinsed again with PBS prior to imaging.

In order to determine the proportion of rNST neurons which express GAD67, sections from three adult GAD67-GFP knock-in mice were prepared and immunostained with the neuronal marker NeuN (Mullen et al. 1992). Mice were deeply anesthetized, perfused with saline, followed by 4% paraformaldehyde and the brain removed and cryoprotected in 20% sucrose phosphate buffer (PB). Brainstem sections (30 µm) were cut on a freezing microtome and those from the NST were rinsed in phosphate-buffered saline (PBS), non-specific binding sites blocked in 5% donkey serum, and then incubated overnight at room temperature in a primary antiserum against NeuN (Millipore, MAB377, 1:1000 in PB/0.2% Triton X-100), rinsed again in PBS, then incubated in a fluorescently-tagged (AlexaFluor 546) anti-mouse secondary antibody (1:500, Invitrogen), overnight before final rinses in PBS and dilute PB. A section representing
the middle of the rNST along its rostro-caudal axis was selected from each of the three cases and photomicrographs were taken with a laser scanning confocal microscope (Olympus Fluoview 1000) using appropriate lasers and filter settings for viewing the native EGFP (Argon 2/488nm, 480-495nm) and AlexaFluor 546 (HeNe1, 543nm, 550-660). EGFP and NeuN neurons were counted by viewing individual z-levels from confocal stacks taken at 2uM intervals using a 20X lens (NA=.85; 0.621 uM/pixel). Cells positive for NeuN immunofluorescence and for native EGFP were counted separately, and the ratio reported. The neuron densities were calculated from the number of neurons and the area within the rNST borders.

Data Collection and Analysis

ST-evoked amplitude data were collected in 2.2 minutes increments (40 sweeps each) during the baseline (immediately preceding DAMGO application), DAMGO (beginning 3.3 minutes following the onset of DAMGO application), and washout (beginning 6.6 minutes following the return to baseline solution) conditions, and measurements from individual sweeps were averaged within these periods. Although there were typically 40 sweeps in each condition, some data were collected using multiple stimulus intensities and in those cases only 10 sweeps were available from each threshold-defined input (with the time periods remaining the same). Measurements were taken using ClampFit software (Molecular Devices, Sunnyvale, CA) and analyzed using Systat.

The responses to ST stimulation were measured as the maximum amplitude of the evoked current relative to baseline, and the paired-pulse ratio (PPR) was calculated as the ratio of the amplitude of the second response to the first. In cases where an accurate PPR could not be measured during DAMGO application (usually due to near-complete suppression or an increase in the failure rate) the cells were excluded from the PPR analysis. The latency was measured as the time from the peak of the stimulus artifact to the onset of the response, and jitter was calculated as the standard deviation of the latency measurements (Doyle and Andresen 2001). Data were analyzed using t-tests or ANOVA
as appropriate. Significance was defined as $P<0.05$. Where arithmetic means are reported, we provide the standard error of the mean. The PPR analysis was done separately for cells with polysynaptic input (defined as jitter $>300\ \mu s$) due to the potential of a polysynaptic pathway to complicate the measurement of a change in the PPR.

Miniature EPSCs were identified using Mini Analysis software, and their rates and amplitudes were compared across the baseline, DAMGO, and washout conditions. These conditions were defined as two minute segments immediately before DAMGO application (minutes 3-5), three minutes following the onset of DAMGO application (minutes 8-10), and seven minutes following the beginning of washout (minutes 17-19).

**Results**

*A significant proportion of rNST neurons are GAD67+*

In order to estimate the proportion of rNST neurons which are GABAergic, we stained for the neuronal marker NeuN in tissue from three GAD67-EGFP knock-in mice. Overall, GAD67+ neurons accounted for $35.6 \pm 4.8\%$ of rNST neurons. The average density of GABAergic neurons was $2400 \pm 540$ cells/mm$^2$ and of NeuN labeled neurons was $6700 \pm 660$ cells/mm$^2$. There were no obvious differences in the expression of GAD67+ across rNST subdivisions (Fig. 1). We took advantage of the broad distribution of GAD67-EGFP expression to record from both GAD67+ GAD67- neurons throughout the nucleus (Table 1).

*A majority of both GAD67+ and GAD67- neurons receive monosynaptic ST input*

Across the entire population, responses in GAD67+ and GAD67- neurons had, respectively, average latencies of $2.41 \pm 1.49$ ms and $2.11 \pm 0.79$ ms ($p = 0.34$) and average jitter values of $290 \pm 381\ \mu s$ and $261 \pm 239\ \mu s$ ($p = 0.74$). Although a jitter of less than $200\ \mu s$ has often been used as a criterion for monosynaptic input to cNST
(Doyle and Andresen 2001), in the present study we used a value of 300 µs (Fig. 2) because a recent study demonstrated that rNST neurons with anatomically-defined direct ST inputs can have jitters that reach this value (Wang and Bradley 2010b). Based on a jitter criterion of less than 300 µs, most ST-evoked responses in rNST neurons were monosynaptic both in the adult (78%) and pup (80%) populations. Likewise, most ST-evoked responses were monosynaptic in both GAD67+ (81%) and GAD67- (79%) neurons. It is important to note that since we made an effort to isolate the lowest threshold response from each cell, our recordings were likely biased towards monosynaptic inputs because polysynaptic inputs would have to overcome the threshold of an interneuron to be detectable.

A small subset of biocytin-filled neurons (n=4) were recovered and the sections immunostained for P2X2r. All of these neurons received monosynaptic inputs (defined as jitter < 300 µs) and three of four were located in the P2X2r terminal field, presumably corresponding to the rostral central (RC, (Whitehead 1988)) subnucleus, whereas the remaining neuron was ventral to the P2X2r staining. In fact, we recorded a number of low-jitter, presumably monosynaptic responses in a similar ventral location, corresponding to the position of the ventral subdivision (Table 1; Fig. 3).

**DAMGO suppresses ST-evoked input to rNST neurons**

Across the population (n=54), DAMGO had a generally suppressive effect on ST-evoked responses save for one response that increased by 53% over baseline. The suppression was reversible in many individual cells and across the population (Fig. 4A, B). For the remaining 53 responses, the mean suppression was 46 ± 3.6%, and this effect was nearly identical in pups and adults (45% and 48%, respectively). Nevertheless, there was considerable heterogeneity in effect size, ranging from nearly total suppression to virtually none (Fig. 4C).

In the pooled data (Fig. 4B), suppression by DAMGO did not vary between GAD67+ and GAD67- neurons (p = .188). Closer scrutiny, however, suggested that GAD67+ and GAD67- neurons were differentially sensitive to DAMGO depending whether they were
under inhibitory blockade. It was possible to make this comparison directly using the data collected in pups, since DAMGO was applied in both ACSF and gabazine/strychnine (GS) baseline conditions. When the slice was bathed in ACSF, GAD67- neurons were significantly more suppressed (57.3%) compared to GAD67+ neurons (34.6%) but when the slice was bathed in GS, GAD67+ neurons showed more suppression (55.8%) compared to GAD67- neurons (38.5%) (ANOVA, DAMGO X inhibitory blockade, p=.006). The greater suppression in GAD67+ neurons compared to GAD67- neurons was consistent in the adult experiments which were all performed in the presence of GS (suppression in GAD67+ 63.5% vs. GAD67- 29.6% (p = .003)). Figure 4D shows the difference between DAMGO’s effects on GAD67+ and GAD67- neurons as a function of time for the GS group (pups + adults).

**DAMGO has a presynaptic site of action**

The paired-pulse ratio (PPR) is calculated as the second response to stimulation divided by the first, and reflects the use- and time-dependent short-term plasticity of transmitter release from the presynaptic terminal (Zucker and Regehr 2002). Many rNST neurons displayed significant trial to trial variability in their response to ST stimulation and this variability allowed us to visualize the relationship between the amplitude of the first response and the paired-pulse ratio within single cells, as shown in the example in figure 5B. When the first response was smaller, the PPR tended to be larger, possibly due to the effects of residual calcium in the terminal coupled with a smaller degree of vesicle depletion (Debanne et al. 1996). Conversely, when the first response was larger, the paired-pulse ratio tended to be smaller. This relationship yielded a characteristic amplitude vs. PPR plot, and in the presence of DAMGO, the points shifted so that as the amplitude of the first pulse decreased, the PPR increased.

This kind of change in the paired-pulse ratio accompanying a change in the response amplitude typically indicates a presynaptic site of action, while an unchanged paired-pulse ratio is indicative of a postsynaptic effect (Zucker and Regehr 2002). For monosynaptic responses (n=44) we observed a significant increase in the PPR in
conjunction with DAMGO suppression (mean increase = 0.166, P<.001). This was true for both GAD67+ (mean increase = 0.195, P=0.004) and GAD67- neurons (mean increase = 0.127, P=0.035) (Fig. 5C). There were no differences between adults and pups or whether the bathing solution was ACSF or GS in the increase of the PPR under DAMGO.

For the population of monosynaptic responses, the magnitude of the PPR change was positively correlated with the magnitude of DAMGO suppression (Pearson’s r=0.396, P=0.005). In other words, the more the first response was suppressed by DAMGO, the greater the relative increase in the second response. Polysynaptic responses (n=10) were analyzed separately because of their potential to complicate the measurement of PPR changes. Although polysynaptic responses did show an increase in the PPR accompanying suppression by DAMGO, the change was not statistically significant (P=0.096).

Monosynaptic ST-evoked responses in GAD67+ neurons had a mean PPR (0.92) which was significantly less than that of GAD67- neurons (1.19) (P < .03). That is, with a 33 ms interstimulus interval, ST input to GAD67+ neurons displayed short-term depression compared to short term facilitation for GAD67- neurons. This was the case despite a tendency for GAD67-neurons to have nominally larger ST-evoked responses (P=0.36), which would if anything be likely to give rise to a smaller paired-pulse ratio (Fig. 5D).

In order to confirm the presynaptic site of action, we recorded miniature EPSCs in 7 rNST neurons from adult animals (2 GAD67+ and 5 GAD67-) during the application of DAMGO. The average baseline frequency was 1.89 + 1.06 Hz. and the average amplitude was 20.6 + 2.9pA. DAMGO decreased the average frequency to 1.08 + 0.53Hz. (Fig. 5E, p=0.014) but did not significantly alter the average amplitude (20.3 + 2.4 pA, Fig. 5F, p=0.54). This is consistent with a presynaptic mechanism of action, since the frequency of release was altered while the postsynaptic response to a quanta of neurotransmitter remained unchanged.
**DAMGO directly hyperpolarizes some rNST neurons**

We observed infrequent (n=5) postsynaptic effects of DAMGO in rNST. In all five cases, the holding current (at Vhold = -70 mV) became more positive (-22.6 + 7.1 vs. -8.5 + 18.5 pA) and the membrane resistance was reduced (994 + 509 vs. 612 + 343 MΩ) with the application of DAMGO. These effects were reversible with washout, and are consistent with the direct activation of an inhibitory conductance. Aside from these five cells, membrane resistance in all cells remained on average within 22% of baseline levels throughout DAMGO application.

**All DAMGO responses were abolished by the µ-opioid antagonist CTAP**

We tested the activity of DAMGO in the presence of the µ-opioid receptor antagonist CTAP in neurons from adult animals (4 GAD67- and 1 GAD67+). No DAMGO effects on amplitude (P=0.95) or PPR (P=0.4) were observed in any of the CTAP control cells (Fig. 4c; Fig. 6), indicating that the responses to DAMGO were due to specific activation of µ-opioid receptors.

**Discussion**

The results of the present study demonstrate that the MOR agonist DAMGO has a suppressive effect on ST-evoked rNST responses that acts primarily through a presynaptic mechanism on both GAD67+ and GAD67- neurons. Though both types of neurons received substantial monosynaptic input, we observed that they differed in their response to a paired-pulse stimulation protocol. At the stimulus frequency tested, ST input to GAD67+ neurons had a smaller paired-pulse ratio than did input to GAD67- neurons, reflecting a tendency for inputs to GAD67+ neurons to exhibit more short-term depression while inputs to GAD67- neurons exhibited more short term facilitation.
The GAD67-EGFP knock-in mouse

The original report of the knock-in mouse model used in this study compared immunohistochemistry for GAD67 and GABA to EGFP expression in neocortical neurons and reported nearly complete overlap of EGFP with both GABA itself and the synthetic enzyme (Tamamaki et al. 2003). Thus it would appear that the use of this mouse is a more effective model for comparing properties of GABAergic with non-GABAergic neurons than are some other commercially available strains. For example, the “GIN” mouse expresses EGFP mainly in somatostatin GABAergic neurons in the neocortex (Oliva et al. 2000), and in the rNST, EGFP expression is confined to just half of the GABAergic cells (Wang and Bradley 2010a) and is particularly abundant in the ventral subnucleus (Travers et al. 2007; Wang and Bradley 2010a). This preferential distribution contrasts sharply with the homogenous pattern observed in the GAD67-EGFP knock-in mouse in the present investigation (Fig. 1). Nevertheless, co-expression of GABA and EGFP has not yet been evaluated in NST and it remains possible that there is a subpopulation of GABAergic neurons in the GAD67-GFP knock-in that are not evident. For example in the olfactory bulb, although some GABAergic neurons express both GAD67 and GAD65, some only express one of the two isoforms (Parrish-Aungst et al. 2007).

The proportion of rNST neurons expressing GAD67

Previous estimates of the proportion of rNST neurons which are GABAergic have been based on GABA immunoreactivity in the CT terminal field of rats (18%, (Lasiter and Kachele 1988)) or on counts of GFP-expressing neurons in the ventral subdivision using the “GIN” mouse (24%, (Wang and Bradley 2010a)). We report a proportion of approximately 36% throughout the nucleus in the GAD67-EGFP knock-in mouse, which is notably higher than previous studies. This perhaps reflects the strength of using a genetic model to label the GABAergic neurons, but could also reflect differences between rats and mice or between the subdivisions of the rNST.
**ST stimulation evokes monosynaptic responses in GABAergic rNST neurons**

We recorded from an approximately equal number of GAD67+ and GAD67- neurons in rNST, and observed no differences between the latency and jitter of these two groups. This was true in the mouse pups both in normal extracellular ACSF and when we added gabazine and strychnine to remove any confounding effect of evoked inhibition. The same experiment in adults verified that the lack of a difference in latency and jitter was not dependent on development since the latency and jitter measurements of all four populations (GAD67+/-/ in adult/pup) overlapped extensively (Fig. 2). These results are similar to those from the cNST, where 50-70% of GABAergic neurons have been shown to be monosynaptically innervated by the ST (Bailey et al. 2008; Glatzer et al. 2007). However, our report that a high proportion of rNST GAD67+ neurons with monosynaptic input appears at odds with a recent anatomical report (Wang et al. 2012) which showed that only a small fraction of identified CT afferent fibers make synaptic contact with GABAergic neurons in the adult rat. It is important to note that we report the percentage of GABAergic neurons receiving monosynaptic ST input, while Wang et al. report the percentage of CT terminals which synapse onto GABAergic neurons. These are not necessarily mutually exclusive observations; it is possible, for example, that non-GABAergic neurons receive much denser innervation from the ST, which would reconcile the observations. In addition, there are no available anatomical data on the relationship between NST GABAergic cells and glossopharyngeal afferents, another population of fibers we undoubtedly stimulated. Moreover, the difference between the studies could reflect a species difference in innervation pattern.

Monosynaptic input to inhibitory rNST neurons implies a mechanism of feed-forward or lateral inhibition in the nucleus at the level of the primary afferent synapse. Although the function of this inhibition is unknown, results from *in vivo* recording studies suggest that GABAergic inhibition in the rNST may sharpen chemosensitive tuning profiles (Smith and Li 1998). One can imagine multiple configurations of afferent inputs and first-order neurons that could underlie such a function but more information about the distribution and convergence patterns of individual afferents will be required in order
to define a more precise role for these monosynaptic inputs to inhibitory rNST neurons in taste processing.

*Short term synaptic plasticity*

The paired-pulse ratios that we report at an interstimulus interval of 33 ms are considerably higher than those that have been previously reported in the rNST, even at similar ISIs (Zhu et al. 2009, Wang #2131). Since the paired-pulse ratio can be significantly affected by experimental conditions that are typically not held constant between studies, most notably stimulus frequency (Zucker and Regehr 2002) and temperature (Klyachko and Stevens 2006), it is difficult to make a direct comparison between different studies. Nevertheless, we frequently observed facilitatory responses, which have not been reported in the rNST *in vitro*, but are consistent with an *in vivo* report showing that a subpopulation of rNST neurons responds with significant facilitation to paired-pulse stimulation of the glossopharyngeal nerve (Hallock and Di Lorenzo 2006). Under the experimental conditions of the present study (33 ms interstimulus interval at 36°C), GAD67- neurons received inputs characterized by larger (facilitatory) paired-pulse ratios compared to inputs received by GAD67+ (GABAergic) neurons.

That these GAD67-, perhaps glutamatergic neurons have a “facilitatory bias” could imply a more linear transformation of the sensory input compared to the depressive response in GAD67-EGFP positive neurons (MacLeod 2011). Future work characterizing the frequency dependence of the PPR in identified neurons as well as their projection status might shed light on the function of this difference in short term synaptic plasticity.

*DAMGO (presynaptically) suppresses ST-evoked responses in the rNST*

Based on the observations that DAMGO reduced the paired-pulse ratio of evoked responses and the frequency, but not the amplitude, of miniature EPSCs it seems likely
that DAMGO acts mainly presynaptically to suppress primary afferent input by reducing release probability. MORs have not been demonstrated specifically on identified gustatory afferents but there is nevertheless strong support for an anatomical basis for this effect. MORs exist in the petrosal, as well as the nodose and jugular ganglia (Ding et al. 1998), although their expression has not been studied in the geniculate. In addition, several published reports show photomicrographs of MOR staining in incoming solitary tract afferents at the level of the rNST (Li et al. 2003; Monteillet-Agius et al. 1998; Moriwaki et al. 1996) and electron microscopic studies report the receptor on axon terminals in the rostral part of the nucleus (Huang et al. 2000).

In the present study, presynaptic inhibition mediated by MORs was observed in young and adult animals and in both GAD67+ and GAD67- neurons. Presynaptic inhibition mediated by MORs occurs for other select types of primary afferents: nociceptive Aδ and C fiber projections to the spinal cord e.g. (Heinke et al.) as well as vagal visceral inputs to diverse neuron types in the cNST including GABAergic (Glatzer et al. 2007; Glatzer and Smith 2005), A2 catecholaminergic (Cui et al.) and POMC (Appleyard et al. 2005) neurons. MOR-mediated presynaptic inhibition also occurs for intrinsic inhibitory and excitatory synaptic transmission in the cNST (Glatzer and Smith 2005).

Although presynaptic inhibition was evident for both GAD67+ and GAD67- cells, the magnitude of suppression was a function of both GAD67-EGFP status and the presence of inhibitory blockade. Presynaptic inhibition was stronger for GAD67-neurons in ACSF but stronger for GAD67+ neurons when GABA\textsubscript{A} and glycine receptors were blocked. These results imply an interaction between the effects of µ-opioid and inhibitory amino acid receptors in rNST. Similar interactions are common in other parts of the CNS, such as the rostral ventromedial medulla (Pedersen et al. 2011) and ventral tegmental area (Johnson and North 1992). The fact that inhibitory blockade preferentially increases the impact of DAMGO on GAD67+ neurons hints that inhibition in the rNST may affect GABAergic and non-GABAergic neurons differently, and that differences could be systematically related to DAMGO sensitivity. Additional evidence,
including recordings from the same cells before and after inhibitory amino acid receptor blockade, would be useful in verifying this hypothesis.

DAMGO also appears to directly inhibit a small proportion of rNST neurons by increasing a hyperpolarizing conductance, consistent with reports of postsynaptic effects of MOR activation in cNST (Poole et al. 2007; Rhim et al. 1993). We saw this effect infrequently, however, and when we did it was typically small. It is conceivable that calcium chelation by EGTA in our recording pipettes could have prevented or reduced any calcium-dependent postsynaptic effects of DAMGO, but this seems unlikely in light of the postsynaptic DAMGO effects seen by other groups using the same concentration of intracellular EGTA (Poole et al. 2007; Rhim et al. 1993). It is also possible that if we took steps to maximize the effect of a small inhibitory conductance change we would observe an enhanced postsynaptic response more frequently. At any rate, MOR mediated inhibition in the rNST appears similar to that observed in the cNST where activation of MORs presynaptically suppress afferent input to many second-order neurons (Appleyard et al. 2005; Cui et al. 2012; Glatzer et al. 2007; Poole et al. 2007) but also has a postsynaptic inhibitory influence on a more restricted population (Poole et al. 2007).

Functional Significance of MOR modulation in rNST

Opioids modulate a wide range of visceral and autonomic processes in the brainstem. In the rNST, varicose fibers containing endogenous ligands for MORs are plentiful, including the enkephalins, which activate δ- as well as μ-opioid receptors (Raynor et al. 1994) and the endomorphins, which are specific MOR agonists (Zadina et al. 1997). The source of these fibers is not certain but likely origins include the cNST and hypothalamus, regions compatible with a role for MOR modulation related to visceral state. Enkephalinergic neurons are distributed in a wide variety of CNS regions (e.g., (Fallon and Leslie 1986)), and such cells often project locally (Fields 2004; Llewellyn-Smith et al. 2005; Poulin et al. 2006; Schneider and Walker 2007). Moreover, enkephalinergic neurons are found in the caudal, as well as the rostral NST (Davis and Kream 1993; Fallon and Leslie 1986; Murakami et al. 1987; Stornetta et al. 2001) but
endomorphinergic soma are located only in the cNST at the level of area postrema and the hypothalamus near and in the dorsomedial and arcuate nuclei (Martin-Schild et al. 1999; Pierce and Wessendorf 2000).

Notably, activation of MORs in several forebrain regions such as the nucleus accumbens strongly increase food intake, particularly of palatable substances, e.g., (Pecina and Berridge 2000). In the cNST, MOR activation suppresses ST input to A2 (Cui et al. 2012) and POMC neurons (Appleyard et al. 2005), effects also compatible with an orexigenic influence since these neuronal populations have been implicated in satiety (Rinaman 2011). Although early reports suggested that rNST DAMGO infusions also increased food intake (Kotz et al. 1997), these studies used rather large infusions and the feeding effect was delayed, suggesting that the orexigenic effect may have arisen from ligand spread that suppressed satiety signals in cNST (Kinzeler and Travers 2011). Indeed, although smaller DAMGO infusions in rNST profoundly impacted the sensory-motor response to intraoral infusion of taste stimuli, they did not prolong the response to sucrose which would have been consistent with a MOR induced increase in feeding. Rather, effects were generally suppressive in that both the rate of intraoral ingestive and rejection responses and the bout duration of ororhythmic responses to QHCl and water were reduced. The reduction of ST-evoked responses observed in the present study, particularly in GAD67- neurons, forms a likely basis for producing shorter bout durations since bout duration is a positive function of stimulus concentration (Davis 1973; Grill and Norgren 1978) and consequently the magnitude of the evoked rNST response, e.g., (Ganchrow and Erickson 1970). Indeed, in vivo infusions of met-enkephalin into the rNST suppress taste-evoked activity in rNST neurons (Li et al. 2003), although this inhibition probably arises from a combination of postsynaptic inhibition by δ-opioid receptors (Zhu et al. 2009) as well as the MOR mediated inhibition observed in the present study.

Paradoxically, in the behavioral studies, DAMGO infusions also changed a neutral licking response to water to an oral rejection “gape” response typically associated with infusions of bitter stimuli. This effect of DAMGO may reflect a disinhibitory
mechanism. We have previously demonstrated that infusions of the GABA$_A$ antagonist, bicuculline, into the reticular formation and overlying rNST produced large amplitude gapes in response to taste stimuli that normally elicited small amplitude mouth openings; i.e., gapes are produced by suppressing GABAergic activity (disinhibition) (Chen and Travers 2003). A disinhibitory interaction between MOR and GABAergic neurons in cNST has also been postulated to account for increased excitability in vago-vagal reflexes (Herman et al. 2009). Thus, we propose that MOR suppression of ST-evoked responses in GABAergic rNST neurons could, in principle, mediate the large amplitude gape responses to water stimulation observed following DAMGO infusions into the rNST (Kinzeler and Travers 2011) by a similar mechanism of MOR-mediated disinhibition.

Overall, activation of MORs in the rNST has behavioral effects that could arise from both inhibitory and disinhibitory mechanisms, consistent with our findings that both GAD67+ and GAD67- neurons receive DAMGO-sensitive monosynaptic input from the solitary tract. The fact that MORs modulate incoming orosensory signals to GABAergic as well as to putative excitatory rNST neurons underscores the complexity of processing in this first-order gustatory relay.
Figures

Figure 2.1.
Confocal images of a section from a level of the rNST in an adult GAD67+ mouse approximately mid-way between the rostral pole of the nucleus and where the NST abuts the IVth ventricle. Sections were also immunostained for the neuronal marker, NeuN. A & B. Low-power maximum-intensity projections showing the distribution of GAD67+, EGFP-stained neurons and fibers (green, A) and EGFP expression merged with NeuN staining (magenta, B). Arrowheads indicate the borders of the nucleus. Note, however, that the most medial pole contains sparse somal label for either marker and corresponds with a region occupied by preganglionic parasympathetic neurons that constitute the rostral pole of the dorsal motor nucleus of the vagus. Aside from this region, GAD67+ neurons are distributed throughout the nucleus; likewise, the nucleus is characterized by profuse EGFP staining of the neuropil. C-E. Higher-magnification images at a single Z plane. Arrows indicate examples of double-stained neurons; arrowheads neurons stained only with NeuN. Scale bars: 250µM (A & B); 25µM (C-E).
Figure 2.1
EGFP Expression in the rNST
Figure 2.2.

Many cells, including GAD67+ neurons, received monosynaptic afferent input from the ST, and this innervation persisted into adulthood.

A plot of latency vs. jitter (A) shows that most responses elicited by ST stimulation had a jitter value below 300 µs. A histogram of jitter values (B) shows the cutoff (dotted line) that we used to define mono- vs. polysynaptic innervation at 300 µs. The inset in (B) shows an example of raw data from a single neuron with an average latency of 1.69 ms and a jitter of 150 µs.
Figure 2.2
Latency and Jitter

A.

B.
Figure 2.3.

Photomicrographs of a ventrally-located site where a GAD67+ neuron was recorded. A. Low magnification DIC image showing the stimulating electrode on the solitary tract (ST) and recording electrode in the ventral 1/3 of the nucleus; the position of the tip is indicated by “*”. The approximate outlines of the rNST are indicated by the dotted line. Pieces of debris (x) cut across the medial part of rNST and medial vestibular nucleus (MeV, x). PCRt (parvocellular reticular formation). B. High magnification DIC image showing the pipette on the recorded neuron. C. High magnification fluorescent image showing that the cell is GAD67+. Scale in A = 250µm; B and C=25µm.
Figure 2.3
Recording Location
Figure 2.4.

DAMGO suppressed the response of rNST neurons to ST-evoked input. A. Effect of DAMGO on the amplitude of a ST-evoked current in a single GAD67- neuron under gabazine/strychnine (GS) blockade in a pup. ST-evoked responses were measured at 3.33s intervals. B. Mean (± S.E) amplitudes of rNST responses in GAD67+ and GAD67- neurons across the population before, during, and after DAMGO application (n=53; the one response facilitated by DAMGO was omitted). ANOVA indicated an overall effect of DAMGO (P<.0005), but no effect of GAD67-EGFP status or a DAMGO X GAD67-EGFP interaction; Bonferroni adjusted post-hoc comparisons revealed significant differences between all 3 conditions: baseline, DAMGO, and washout (all P’s<.005). C. Dot density plot showing the degree to which individual responses were suppressed by DAMGO (response increment omitted). Responses are grouped by animal age and the bath solution: artificial cerebral spinal fluid (ACSF), gabazine and strychnine (GS), gabazine and strychnine with the MOR antagonist CTAP (CTAP). Symbols indicate monosynaptic vs. polysynaptic responses and GAD67-EGFP status. D. Effect of DAMGO shown for normalized responses over time for GAD67+ (n=15) and GAD67- (n=15) neurons recorded when gabazine and strychnine were included in the bath. Individual measurements are for the moving average of 5 adjacent time points. When inhibitory amino acid receptors were blocked, DAMGO had a significantly larger effect on ST-evoked responses in GAD67+ than in GAD67-neurons, though responses in both cell types were affected.
Figure 2.4
The Effect of DAMGO on ST-Evoked EPSCs
The effect of DAMGO on rNST neurons is presynaptic. A. The response of an rNST neuron to ST stimulation (averages of 40 sweeps in voltage clamp) before, during, and after the application of 0.3 µM DAMGO. B. Single data points showing the relationship between the amplitude of the first response to ST stimulation and the PPR for the same cell in each condition (baseline, DAMGO, and washout). C. Across the population, the average PPR increases during DAMGO application for both GAD67+ and GAD67- neurons. D. Scatterplot of average baseline amplitude and PPR values for individual monosynaptic ST-evoked responses (n=44) showing an inverse relationship between the first response amplitude and paired pulse ratio for both GAD67+ and GAD67- neurons. The paired pulse ratios of monosynaptic responses to ST stimulation were significantly smaller in GAD67+ neurons than in GAD67-neurons. This effect may be seen most clearly in the frequency polygons (binned in 15 equal segments) displayed outside the graph area; scale bars correspond to a frequency of 10 neurons. E. The frequency of miniature EPSCs (tested in 7 neurons, 2 GAD67+ and 5 GAD67-) was significantly reduced by DAMGO. F. The average amplitude of miniature EPSCs was unaffected by DAMGO. Taken together, these results indicate a presynaptic site of action.
Figure 2.5
The Effect of DAMGO is Presynaptic
All DAMGO effects are abolished in the presence of the µ-opioid antagonist CTAP. Mean (A) amplitudes of evoked responses and (B) paired-pulse ratios from 5 neurons recorded under MOR receptor blockade accomplished by adding 1 µM CTAP to a bathing solution containing normal ACSF with gabazine (5µM) and strychnine (5µM). Under these conditions, no significant change in the amplitude of the evoked response or PPR was observed when 0.3 µM DAMGO was added, indicating that DAMGO effects in the absence of CTAP blockade were specifically mediated by MOR receptors.
Figure 2.6
DAMGO Effects are Abolished by CTAP
Chapter 3: A Mathematical Model of Gustatory Processing in the Rostral Nucleus of the Solitary tract

Abstract

Primary gustatory afferents synapse onto central neurons in the rostral nucleus of the solitary tract (rNST), and both peripheral fibers and central rNST neurons respond to gustatory stimuli with an increase in firing frequency which varies between stimuli within individual units. The breadth of tuning describes the specificity of these responses, and rNST neurons are typically more broadly tuned and respond with higher frequencies than afferent fibers. This transformation might result in part from central convergence of gustatory afferents onto rNST neurons.

In order to systematically evaluate the degree to which individual features of the rNST such as convergence contribute to signal processing, we created a computational model of transmitter release coupled to a conductance based model of an rNST neuron which was fit to in vitro measurements of release and postsynaptic excitability. We recorded synaptic currents evoked in rNST neurons by electrical stimulation of the ST in an in vitro slice preparation and found that although ST-evoked EPSCs were characterized by significant short term synaptic depression (STSD), the relationship between input frequency and total evoked postsynaptic conductance was an increasing, saturating function over the range of frequencies tested. We injected small steps of current in order to quantify the relationship between applied current and postsynaptic firing rate, and found that rNST neurons were characterized by a nearly linear
relationship between applied current and firing frequency, followed by a decrease with
the application of additional current (“depolarization block”), underscoring the
importance of keeping the input within the dynamic range of these neurons. This in vitro
work was performed in a mouse model expressing VENUS under the control of the
VGAT promoter, and this allowed us to compare the responses of inhibitory and putative
excitatory neurons. We found that inhibitory neurons have significantly smaller ST-
evoked synaptic currents, nominally lower thresholds, and go into depolarization block at
significantly smaller applied currents. Maximal firing frequencies were not different
between the two groups.

We fit our mathematical model to the average characteristics of the excitatory and
inhibitory rNST neurons recorded in vitro, resulting in an excitatory and an inhibitory
rNST neuron model. We used rates derived from existing in vivo single unit chorda
tympani recordings as the input to these models and compared their output frequencies to
rNST responses recorded under similar conditions, and we found that a random pattern of
2-10 convergent afferents produced modeled rNST responses which were more broadly
tuned than those recorded in vivo. More selective patterns of convergence (e.g. all
sucrose-best fibers), resulted in more narrowly tuned responses which were a better fit to
the in vivo data. Tonic inhibition is thought to play a role in maintaining the specificity
of rNST responses, and the addition of various forms of inhibition to our mathematical
models sharpened the breadth of tuning and improved the fit of the model to the in vivo
data. Specifically, presynaptic inhibition sharpened the breadth of tuning of the
excitatory but not the inhibitory model, postsynaptic inhibition sharpened the breadth of
tuning of both modeled neuron types, and synaptic inhibition from the inhibitory to the
excitatory model sharpened the breadth of tuning of the excitatory model only when the
inhibitory model received random, broadly tuned input (a “center-surround”
configuration). We predict that one or all of these mechanisms is tonically active in the
rNST.
**Introduction**

As taste information passes from peripheral afferents to the central nervous system it must pass through the rostral nucleus of the solitary tract (rNST) before being relayed to either ascending pathways via the parabrachial nucleus or local reflex pathways in the reticular formation. It is not well understood how taste information passing through the rNST is transformed by the intrinsic properties of the network, although the inputs, outputs, and many of the synaptic and neuronal mechanisms have been characterized (reviewed in Spector & Travers, 2005).

Peripheral afferents respond to the application of each of four or five tastants (sweet, salty, sour, bitter, umami) with an increased frequency of action potentials, and the specificity of this response varies between afferents. The breadth of tuning refers to some measure of this specificity, and while some afferents are broadly tuned and respond well to multiple tastants, others are narrowly tuned and respond primarily to one with a minimal response to the other three. The most commonly used measure of this specificity is entropy (H) (J. B. Travers & Smith, 1979), but an additional useful metric is the noise-to-signal ratio (R) (Spector & Travers, 2005). The firing frequencies and breadth of tuning of peripheral afferents, as well as central rNST neurons, have been investigated in vivo in different species using different experimental conditions, but few data sets exist which compare peripheral and central responses under the same experimental conditions, including species, stimulus concentrations, flow rates and duration of stimulus application, and anesthetic (Frank, 1973; Ganchrow & Erickson, 1970; J. B. Travers & Smith, 1979). In Travers and Smith (1979) the authors found that both firing frequency and breadth of tuning are modestly increased in the hamster rNST neurons compared to peripheral chorda tympani (CT) afferents (Frank, 1973).

These increases are thought to be a result of convergence of peripheral gustatory afferents onto rNST neurons (J. B. Travers & Smith, 1979). Very little is known about the specific rules governing convergence between gustatory afferents. Possibilities range from entirely random central convergence to selective convergence of only very narrowly
tuned afferents of the same best-stimulus type, and different rules may apply to subpopulations of rNST neurons.

The extent to which convergence of any type will alter central firing rates and breadth of tuning depends not only on the degree and pattern of convergence, but also on the intrinsic properties of the afferent synapses. Gustatory afferents display a significant frequency dependent short term synaptic depression (STSD), which has been observed both in vivo (Hallock & Di Lorenzo, 2006; Lemon & Di Lorenzo, 2002; Rosen & Di Lorenzo, 2009) and in vitro (M. Wang & Bradley, 2010a). STSD is known to function as a lowpass filter (Fortune & Rose, 2001), which could theoretically result in a profound increase in breadth of tuning by faithfully passing on low-frequency afferent input while preventing the transmission of the high-frequency response of the afferent to its best-stimulus. Alternately, STSD is also known to act as a gain-setting mechanism which can function to keep the inputs to a neuron scaled within its dynamic range (Abbott, Varela, Sen, & Nelson, 1997). This could be an important mechanism for maintaining specificity of breadth of tuning in the gustatory system in the presence of convergent inputs which would otherwise saturate rNST neurons’ firing rates.

Another important factor which shapes the relationship between peripheral input and rNST output frequencies is the intrinsic excitability of rNST neurons. Although there is a significant literature characterizing the intrinsic properties of rNST neurons (R. M. Bradley & Sweazey, 1989; 1992; Du & Bradley, 1996; Suwabe & Bradley, 2009; Suwabe, Mistretta, Krull, & Bradley, 2011; Tell & Bradley, 1994; M. Wang & Bradley, 2010b), no study to date has attempted to precisely describe the relationship between applied current and firing frequency in these neurons. This relationship captures many critical aspects of neuronal excitability in a simple function and has been used as a measure of excitability in other systems (Caillard, 2011; Prinz, Billimoria, & Marder, 2003).

The infusion of the GABA_A antagonist bicuculline into the rNST suppressed in vivo responses to gustatory stimuli in 63% of rNST neurons (Smith & Li, 1998), suggesting that most rNST neurons are under GABA-mediated tonic inhibition. Blocking
this tonic inhibition resulted in a uniform increase in firing rate across tastants and therefore an increased breadth of tuning in these neurons. An estimated 36% of neurons in the rNST are inhibitory (Boxwell, Yanagawa, Travers, & Travers, 2013), and 68-69% of neurons respond to GABA in vitro (Liu, Behbehani, & Smith, 1993; L. Wang & Bradley, 1993). The vast majority of rNST neurons recorded in vitro, including inhibitory neurons (Boxwell et al., 2013), receive monosynaptic input from the solitary tract (Grabauskas & Bradley, 1996; L. Wang & Bradley, 1995b; M. Wang & Bradley, 2010a), and a large proportion also receive polysynaptic ST-evoked inhibition (Grabauskas & Bradley, 1996; L. Wang & Bradley, 1995b).

The goal of this study was to generate a mathematical model of the rNST in order to determine whether the known properties of the system are sufficient to recapitulate the increase in firing frequency and breadth of tuning observed in vivo in the rNST compared to the CT. We generated a mathematical model consisting of one excitatory and one inhibitory neuron, each fit to measurements of intrinsic excitability measured in vitro in identified inhibitory and putative excitatory rNST neurons. Both modeled neuron types received convergent input from modeled ST afferents with STSD and postsynaptic current magnitudes fit to in vitro data. This simple network configuration does not preclude the possibility of more complex architecture, but represents a minimal starting point which accounts for much of what is currently known about the system.

We used frequencies taken from in vivo CT recordings (Frank, 1973) as input to the modeled afferents, and placed different conditions on the number and pattern of convergent afferents to see if any reasonable set of assumptions resulted in modeled output frequencies which match the firing frequencies and breadth of tuning of real rNST neurons recorded in vivo. Since very little is known about convergence of gustatory afferents in the rNST, we hoped to generate testable predictions regarding the number and pattern of convergent afferents which can account for the in vivo data.

Since inhibition can affect both firing frequency and breadth of tuning, and since the in vivo rNST data (J. B. Travers & Smith, 1979) almost certainly reflects the presence of tonic inhibition (Smith & Li, 1998), we assessed the effect of three types of inhibition...
on the input-output relationship of our model: a presynaptic reduction in transmitter release, a tonic postsynaptic chloride current, and synaptic inhibition from modeled inhibitory rNST interneurons.

Methods

In vitro Recording

Animal Model

We used 6-12 week old mice expressing VENUS under the control of the VGAT promoter in order to record from identified inhibitory (GABAergic and glyceinergic) and putative excitatory neurons. This mouse model has a similar expression pattern to the GAD67-EGFP mouse which we have previously worked with (Boxwell et al., 2013) and lacks the “hole” in expression in the rostral central subnucleus which was observed in the GIN strain (J. B. Travers, Herman, Yoo, & Travers, 2007). All experimental protocols were approved by the Ohio State University Institutional Animal Care and Use Committee in accordance with guidelines from the National Institutes of Health.

In vitro Slice Preparation

Acute slices were prepared for electrophysiological recording following anesthetization with isofluorane, decapitation, and rapid removal and cooling of the brain (Boxwell et al., 2013). The blocked brainstem was glued to a ceramic block using cyanoacrylate glue, and 250 µm thick coronal brainstem slices were cut with a sapphire blade on a vibratome (model 1000, Vibratome, St. Lois, MO, USA) in an ice-cold carboxygenated cutting solution containing (in mM) 110 choline, 25 NaHCO$_3$, 3 KCl, 7 MgSO$_4$, 1.5 NaH$_2$PO$_4$, 10 d-Glucose, 0.5 CaCl$_2$. Slices containing the NST rostral to where it moves laterally away from the fourth ventricle were identified and incubated in a carboxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 25 NaHCO$_3$, 3 KCl, 1 MgSO$_4$, 1.5 NaH$_2$PO$_4$, 10 d-Glucose, and 1.5 CaCl$_2$ at 32°C for one hour prior to recording.
In vitro Recording

Slices were transferred to a recording chamber and perfused with 36°C ACSF containing 5 µM gabazine and strychnine at a rate of 1-2 mL/minute. The NST and solitary tract (ST) were visualized with a Nikon E600FN microscope and a bipolar stimulating electrode made of twisted insulated wire was placed on the ST under visual control.

After placement of the stimulating electrode, cells in the rNST were visualized using IR-DIC optics and epifluorescent illumination to identify cells which were either VENUS-VGAT positive or negative. Approximately equal numbers of VENUS positive and negative neurons were targeted throughout the course of the study. Once a neuron was identified, a whole cell patch clamp recording was made using a 4-6 MΩ pulled glass pipette filled with an intracellular solution containing (in mM) 130 K-gluconate, 10 EGTA, 10 HEPES, 1 CaCl₂, 1 MgCl₂, and 2 ATP, at pH 7.2-7.3 and osmolality 285-290 mOsm. An initial seal of greater than 1 GΩ and a membrane resistance of greater than 100 mΩ were inclusion criteria for seal and cell viability. Recordings were made with an A-M systems Model 2400 amplifier and recorded using pClamp software (Molecular Devices, Sunnyvale, CA).

Recording Protocols

ST-evoked currents were evoked by tract stimulation at frequencies between 1 and 60 Hz. over a 500 ms. stimulus period at a frequency of 0.1 Hz. Each frequency was presented between 6 and 9 times in a varied order and responses were recorded in voltage clamp at a holding potential of -70 mV. Threshold was defined as the current intensity that evoked a response 50% of the time, and the experimental stimulus intensity was set at 10 µA above the threshold to ensure a reliable response. In some cases the presence of an additional, higher-threshold evoked response precluded the use of current values 10 µA above threshold, and in these cases the highest current that did not activate the additional input was used.
The response of rNST neurons to small hyperpolarizing and depolarizing 450 ms. steps of current injection (-0.1 nA + 0.01 nA steps) were recorded in current clamp.

Data Analysis

ST-evoked currents were quantified as the total area under the curve of the current waveform relative to baseline, as calculated by clampfit software. Stimulus artifacts were not excluded from the waveform, but since these artifacts are typically very narrow their contribution to this area metric was minimal.

Voltages in the current clamp data were measured over a 50 ms. portion of the current step, after the voltage had come to steady state. Action potentials were automatically detected as 0 mV crossings. In a few cases the steady-state current exceeded 0 mV after the cell had gone into depolarization block. In these cases the threshold for action potential detection was raised above the steady-state and the results were checked by hand to ensure that no action potentials in previous sweeps were missed. The “break point” was defined as the applied current at which the firing frequency peaked, and in the cases where firing frequency plateaued it was defined as the last applied current at which the firing frequency was at its peak.

Statistical Analysis of In vitro Data

Comparisons between excitatory and inhibitory neuron populations were made using a two-sample t-test, with the exception of the response magnitude to ST stimulation. The functions between input frequency and response magnitude were fit to a Hill-type function of the following form, where R is response magnitude and F is input frequency:

\[ R = \frac{R_{\text{max}}}{1 + \left( \frac{F_{50}}{F} \right)} \]

and the parameters Rmax and F50 were compared using a two-sample t-test.
Mathematical Modeling

We created a model of an rNST neuron (see Figure 3.1 for a diagram) consisting of voltage-gated sodium and potassium conductances, a leak conductance, and a slow voltage-gated potassium conductance ($G_{Ks}$) which was necessary to appropriately space the action potentials. In addition, we modeled postsynaptic inhibition as a small tonic chloride conductance. We modeled synaptic input to this neuron as 1-10 independent convergent afferents, each utilizing a three compartment model of short term synaptic plasticity of transmitter release.

We provided input rates to each modeled afferent individually, and the synaptic release models for each afferent operated independently. The input rates were transformed into poisson-distributed interstimulus intervals over 5 second baseline and stimulus periods, and the onset of the stimulus input was jittered by a random value 1-500 ms. in order to minimize the effect of synchronous onset of inputs. Action potentials were detected as 0mV crossings, and the action potential counts over defined periods were taken as the output of the model.

Modeling rNST Neuronal Excitability

We were able to fit a Hodgkin-Huxley type conductance-based neuron model to key features of the in vitro data, where the cell’s voltage is defined by

$$C_m \times \frac{dV}{dt} = (-I_{Na} - I_K - I_{Ks} - I_{leak} - I_{Cl} - I_{syn} + I_{app})$$

and the voltage gated sodium ($I_{Na}$), voltage gated potassium ($I_K$), slow voltage-gated potassium ($I_{Ks}$), leak ($I_{leak}$), and chloride ($I_{Cl}$) currents are of the following form

$$I_{Na} = G_{Na} \times m^3 \times h \times (V - E_{Na})$$
$$I_K = G_K \times n^4 \times (V - E_K)$$
$$I_{Ks} = G_{Ks} \times n^4 \times (V - E_K)$$
$$I_{leak} = G_{leak} \times (V - E_{leak})$$
\( I_{Cl} = G_{Cl} \times (V - E_{Cl}) \)

where the gating variables \( m, h, n \), and \( n_s \) are of the form

\[
m' = \frac{m_{inf}(V) - m}{\tau_m(V)}
\]

\[
(m_{inf}(V)) = \left( 1 + e^{-\left( V - \theta_{minf} \right) / \sigma_{minf}} \right)^{-1}
\]

\[
\tau_m(V) = a_m + b_m \times \left( 1 + e^{-\left( V - \theta_{a\tau m} \right) / \sigma_{a\tau m}} \right)^{-1}
\times \left( 1 + e^{-\left( V - \theta_{b\tau m} \right) / \sigma_{b\tau m}} \right)^{-1}
\]

See Table 1 for parameter values used in the model fit to the \textit{in vitro} data from both the excitatory and inhibitory rNST neurons.

\textit{Modeling Transmitter Release}

Using xppaut software, transmitter release was modeled as a three compartment model (Ermentrout and Terman, 2010) by a system of equations with the following form:

\[
Y' = \frac{-Y}{D} + (X \times S)
\]

\[
X' = \frac{Z}{R} - (X \times S)
\]

\[
Z' = \frac{Y}{D} - (Z / R)
\]

\[
I_{syn} = G_{syn} \times Y \times (V - E_{syn})
\]

where \( X, Y, \) and \( Z \) are the fractions of neurotransmitter in a readily releasable state, in the synaptic cleft, and unavailable for release, respectively. The value of \( Y \) was scaled by a postsynaptic conductance and multiplied by driving force to produce a postsynaptic
current waveform, $I_{syn}$. The rate constants $D$ and $R$ govern the removal of transmitter from the synaptic cleft and resetting of available transmitter, while synaptic release is triggered by the incrementation of the “$S$” variable by a release probability variable:

\[
S = S + PR \\
S' = -k \times S
\]

This process was triggered at time indexes with poisson-distributed interstimulus intervals (ISIs) generated from an input rate variable. For the purposes of fitting the \textit{in vitro} data, this portion of the synapse model was replaced by a function which generated constant ISIs for 500 ms. durations. See Table 1 for parameter values used in the model fit to the \textit{in vitro} data from both the excitatory and inhibitory rNST neurons.

\textit{Generating Input Frequency-Output Frequency Curves}

We ran the complete mathematical model assuming 1 to 10 convergent afferent inputs each receiving an input frequency of 1 to 30 Hz. The average response rate over the 5 second stimulus period was plotted against the input rate to generate the input-output curve.

\textit{Modeling Methods Using In vivo Data}

We ran the complete mathematical model using a baseline rate as the input rate for 5 seconds, then using a stimulus rate for 5 seconds, and counted crossings at -20 mV as action potentials to generate baseline and stimulus period output rates.

Input rates were taken from CT \textit{in vivo} data as follows using a custom-built python script. First, some number of afferents were selected according to best-stimulus criteria from the data set. Next, the baseline rate and the response rate to sucrose were passed into the model as baseline and stimulus input rates, respectively. The output rates were collected, and then the process was repeated using the salt, acid, and quinine
response rates in order to generate output rates for the virtual application of these stimuli. Together these values were entered into a table as a single modeled cell and the process was repeated with a new randomly chosen group of afferents. We generated 25 cells for each combination of convergence number (2, 4, 6, 8, and 10) and best-stimulus category (random, sucrose-best, salt-best, and acid-best). A quinine-best pattern of convergence was not tested because only one quinine-best afferent was recorded in the CT data set.

Statistical Analysis of Modeled Data

In order to compare the modeled rNST neuron responses to the \textit{in vivo} rNST data, we performed a Kolmogorov-Smirnov (KS) test comparing the \textit{in vivo} data and each tested level of convergence (2, 4, 6, 8, 10) as well as all contiguous groups of these levels (eg. 2-6, inclusive). The smallest score was taken as the best fit to the \textit{in vivo} data, and although there is a probability of statistical significance associated with each KS score it is not reported due to the many comparisons made. When there were multiple lowest scores (after rounding to 2 decimal points) the most inclusive range was reported as the lowest.

Comparisons were made between modeled responses (eg. between excitatory and inhibitory models) by performing a paired t-test on the average change (compared to the baseline model) in the frequency, H, or R values at each level of convergence.

Estimating an Upper Bound for Convergence in the rNST

For the purposes of our mathematical model, we assume that convergence of gustatory afferents onto rNST neurons is likely to be sparse, on the order of ten afferents per neuron rather than hundreds or thousands. Although there is no direct evidence for this, we can place an estimated upper bound on the likely degree of convergence in the CT terminal field in the rNST. One CT afferent may diverge to contact an average of fewer than 13 rNST neurons in the mouse (12.5, (Zaidi, Todd, Enquist, & Whitehead, 2008)) and there are on average about 600 afferents in the rat CT (Farbman & Hellekant, 1978). If we assume that there are likely as many or fewer afferents in the CT of the
smaller hamster and mouse (plausible, since there are about 400 CT cell bodies in the
geniculate ganglion of the rat (May & Hill, 2006) and about 250 in the hamster
(Whitehead, McGlathery, & Manion, 1995)), the number of unique CT-rNST contacts
can be calculated to be something less than 7800, which places an upper bound on the
number of rNST neurons receiving CT afferent input.

Assuming an average convergence of 5 reduces this upper bound of 7800 neurons
to 1560, assuming an average of 10 limits it to 780, and assuming an average of 20 limits
it to 390. At some point this calculated upper bound on the number of rNST neurons
receiving CT input becomes implausible, placing an upper bound on convergence in the
rNST. To put the numbers above in perspective, we estimate that there are something
like 2000-3000 neurons in the rat chorda tympani terminal field (average volume =
31×10^6 µm^3 (May & Hill, 2006) or about 50×10^6 µm^3 (Lasiter, 1992) and average
neuron density = 65,000 neurons/mm³ (King & Hill, 1993)). We believe that our
assumption of 10 or fewer convergent afferents is reasonable; certainly it is of the right
order of magnitude.

Results

ST-evoked Currents in rNST Neurons Display Frequency-Dependent Short Term
Synaptic Depression

We recorded postsynaptic ST-evoked currents from 21 inhibitory and 21
excitatory rNST neurons over a 500 ms stimulus duration at frequencies ranging from 1
to 60 Hz. These responses were characterized by short latencies (2.2 ms in inhibitory
neurons and 1.5 ms in excitatory neurons, p<0.03) and low jitter values (0.19 ms in
inhibitory neurons and 0.14 ms in excitatory neurons, p<0.004) and are therefore likely
monosynaptic responses (Boxwell et al., 2013; Doyle & Andresen, 2001; M. Wang &
Bradley, 2010a). In all neurons, these responses were characterized by pronounced
STSD at all frequencies (Figure 3.2A). The relationship between stimulation frequency
and total synaptic input (measured as area under the recorded trace) was an increasing, saturating function for both excitatory and inhibitory rNST neurons (Figure 3.2B). On average, total synaptic input to excitatory rNST neurons was twice as large compared to inhibitory rNST neurons at all frequencies. When a Hill-type function of the form:

\[ R = \frac{R_{\text{max}}}{1 + \left( \frac{F_{50}}{F} \right)} \]

was fit to the relationship between stimulus frequency and response magnitude, the average \( R_{\text{max}} \) was about twice as large in excitatory compared to inhibitory rNST neurons (18.9+/−14.2 vs. 9.5+/−6.8) and this difference was significant (p<0.01). In contrast, the average \( F_{50} \) was nearly the same for both cell types (32.9+/−17.8 vs. 30.7+/−20.1, p<0.72).

**ST-evoked EPSCs are Effectively Modeled by a Three Compartment Model of Transmitter Release**

We generated a three compartment model of transmitter release scaled by a postsynaptic conductance and fit this model to the relationship between input frequency and total synaptic input measured in the *in vitro* data (Figure 3.2B). Since both excitatory and inhibitory neurons had virtually the same \( F_{50} \), we were able to use the same presynaptic mathematical model and altered the postsynaptic conductance parameter in order to fit the magnitude of response observed at 30 Hz in each neuron type. When we fit a Hill-type equation (see above) to the modeled results, the excitatory modeled neuron had a \( R_{\text{max}} \) of 19.2 and a \( F_{50} \) of 31.7 (compare to 18.9 and 32.9 in the *in vitro* data) and the inhibitory modeled neuron had a \( R_{\text{max}} \) of 9.7 and a \( F_{50} \) of 31.7 (compare to 9.5 and 30.7).

**rNST Neurons Fire Slowly at Low Thresholds and Exhibit Depolarization Block at Minimal Levels of Applied Current**

62
We recorded the voltage and firing frequency of 28 inhibitory and 30 excitatory rNST neurons in response to finely graded steps of injected current between -0.1 and +0.2 nA. The average threshold was 0.0026 nA in inhibitory neurons and 0.013 nA in excitatory neurons, and above threshold firing rate tended to increase linearly with additional applied current to some maximal frequency (59.9 Hz. in inhibitory neurons, 57 Hz. in excitatory neurons) before decreasing sharply at a “break point” (0.083 nA in inhibitory neurons, 0.13 nA in excitatory neurons) above which the neuron’s firing rate in response to applied current decreased until it was only capable of firing a few action potentials before going into depolarization block (see examples in Figure 3.3A). Although there was no significant difference between excitatory and inhibitory neurons’ maximal firing frequencies (2.9 Hz., p<0.68), the inhibitory neurons had nominally lower thresholds (0.01 nA, p<0.073) and significantly lower break points (0.047 nA, p<0.005) (see inset in figure 3.3B).

rNST Neuron Excitability is Effectively Modeled with a Simple Hodgkin-Huxley Type Conductance Based Model

We next fit a Hodgkin-Huxley type conductance based neuron model to the relationship between the applied current and the voltage and firing rate measured in vitro for both excitatory and inhibitory rNST neurons. Our modeled neuron included a leak conductance, a voltage gated sodium conductance, a fast voltage gated potassium conductance, and a slow voltage gated potassium conductance. We were able to fit this model to the behavior of the excitatory and inhibitory in vitro data sets by altering the leak conductance reversal potential (-54mV in the inhibitory model neuron, -59.5mV in the excitatory model neuron) to fit resting membrane potential and threshold and the slow potassium conductance (0.0015 uS in the inhibitory model neuron, 0.003 uS in the excitatory model neuron) to fit the break point. The fit of the model compared to the threshold, break point, and maximum firing frequency of the in vitro data can be seen in Figure 3.3B, and all parameters are listed in Table 1.
The Frequency Input-Output Relationship is Similar in Modeled Excitatory and Inhibitory rNST Neurons, and Increases with Increasing Numbers of Convergent Afferents

Despite the two-fold difference in total synaptic drive and differences in postsynaptic excitability between the modeled excitatory and inhibitory rNST neurons, their relationships between input and output firing frequencies were markedly similar across the tested range of convergent afferents and input frequencies. In both modeled neuron types, the input-output relationship was an increasing, saturating function over the range of physiologically relevant input frequencies, and the slope of this relationship increased with increasing convergence (Figure 3.4A).

When STSD was removed from the modeled synapse (by allowing near-instantaneous resetting of available transmitter, R=2ms) the summed synaptic input rapidly saturated the dynamic range of the postsynaptic neuron. For modeled neurons receiving more than four convergent afferent inputs, the result was a “crash” of the output frequency at higher input frequencies (Figure 3.4B).

The Firing Frequency to Best Stimulus and Breadth of Tuning Are Modestly Increased in the rNST Compared to the CT In Vivo

We used input rates derived from in vivo chorda tympani (CT) recordings in hamster (Frank, 1973) in order to determine how well our complete mathematical model could transform these rates into responses comparable to those recorded in the rNST in vivo (J. B. Travers & Smith, 1979). Both in vivo data sets include a baseline rate as well as a response rate to a five second application of sweet, salty, sour, and bitter stimuli. By plotting firing frequency to best stimulus as well as breadth of tuning for both data sets, it is possible to visualize the increases in firing frequency and breadth of tuning reported in the original work (Figure 3.5).

In S-best units, the average firing rate to sucrose decreased (CT, rNST: 22.2, 17.5 Hz.), while the average breadth of tuning increased (H:0.43, 0.64, R: 0.32, 0.39). In N-best units, the average firing rate to salt increased (19.3, 23.4 Hz.), as did the average
breadth of tuning (H: 0.64, 0.73, R: 0.45, 0.53). In A-best units, the average firing rate to acid roughly doubled (16.5, 33.8 Hz.), while the average breadth of tuning did not change substantially (H: 0.75, 0.73, R: 0.57, 0.60). Overall, both the firing rate and breadth of tuning modestly increased in the rNST compared to the CT data, and the change in frequency was primarily driven by the acid-best population while the change in breadth of tuning was primarily driven by the sucrose- and salt-best populations.

Random Patterns of Gustatory Afferent Convergence Do Not Reproduce In vivo Data

In order to generate a modeled rNST neuron response to compare to the in vivo rNST data, we first selected some number (2, 4, 6, 8, or 10) of CT afferents at random from the in vivo data set. Using this subset of the CT data as input rates to the appropriate number of independent modeled afferents, we ran the mathematical model for a five second baseline period followed by a five second stimulus period. We repeated this using the CT response rates for each tastant in turn, resulting in a modeled rNST response with baseline and tastant response rates for comparison to the in vivo rNST data set.

Under these conditions, a significant majority of modeled rNST neurons were salt-best (76%), in contrast to the much smaller proportion of salt-best neurons in the in vivo rNST data set (30%). In these modeled neurons (Figure 3.6A), the model was a reasonably good fit to the in vivo data with respect to frequency (Excitatory model: KS=0.29, 2-10 afferents, Inhibitory model: KS=0.34, 2-10 afferents) and this can be seen in the frequency portion of Figure 3.6A as the extensive overlap of the box plots representing the model results with the dark gray box plot representing the rNST data. The fit with respect to breadth of tuning was significantly worse, both as measured by H (E: KS=0.54, 4 afferents, I: KS=0.57, 2-4 afferents) and as measured by R (E: KS=0.44, 2 afferents, I: KS=0.39, 2 afferents). This is reflected in the failure of the modeled results in Figure 3.6A to significantly overlap with the dark gray boxes representing the rNST data; the modeled output resulting from random convergence is uniformly too broadly tuned.
Stimulus-Specific Patterns of Convergence Adequately Recapitulate Most In vivo Data

Although random best-stimulus convergence may account for some very broadly tuned rNST neurons, we next tested the hypothesis that stimulus-specific convergence might account for the relative specificity of the majority of the in vivo rNST data. We ran the mathematical model again, this time randomly selecting CT afferents from one of three groups: S-best, N-best, and A-best. Since there was only one Q-best CT afferent in the in vivo data set, we excluded it from this part of the study.

The resulting modeled rNST neurons more closely matched their in vivo rNST counterparts in both firing frequency to best-stimulus and breadth of tuning. Modeled S-best rNST responses fit the in vivo data about as well with respect to frequency (E: KS=0.31, 2-4 afferents, I: KS=0.40, 2-10 afferents) and much more closely with respect to breadth of tuning, both as measured by H (E: KS=0.21, 2 afferents, I: KS=0.21, 2 afferents) and as measured by R (E: KS=0.24, 2-6 afferents, I: KS=0.25, 2-8 afferents). This can be seen as a much better overlap of the R and H values with the rNST data (red bar, Figure 3.6B).

Modeled N-best rNST responses also fit the rNST data about as well as random convergence with respect to frequency (E: KS=0.29, 2-6 afferents, I: KS=0.31, 2-4 afferents). N-best convergence was a better fit with respect to H than random convergence, but not quite as good as S-best convergence (E: KS=0.34, 6-10 afferents, I: KS=0.31, 2 afferents). This can be seen as the partial overlap of the modeled H results with the blue rNST data in Figure 3.6C. The fit of the modeled results with respect to R was just as good as that for S-best convergence and a substantial improvement over random convergence (E: KS=0.23, 2-10 afferents, I: KS=0.23, 2 afferents).

Modeled A-best rNST neurons were a worse fit to the in vivo data than the two other best-stimulus types, failing to account for a population of fast firing in vivo responses (E: KS=0.38, 2-10 afferents, I: KS=0.38, 2-10 afferents). This can be seen as a
large portion of the rNST frequency data (green bar, Figure 3.6D) which is not overlapped by the modeled results. The modeled results also fail to recapitulate a small group of more narrowly tuned A-best cells, resulting in a worse fit than the other two best-stimulus types both with respect to H (E: $KS=0.48$, 6-8 afferents, I: $KS=0.50$, 2-10 afferents) and R (E: $KS=0.31$, 4-10 afferents, I: $KS=0.39$, 10 afferents). These fits to the in vivo data with respect to breadth of tuning are still an improvement over those obtained by assuming random convergence.

A somewhat surprising feature of these results is that increasing convergence from 2 to 10 afferents does not substantially increase the mean entropy of the resulting responses (average H across all three best-stimulus types is 0.78, 0.81, 0.81, 0.81, 0.79), and in fact the mean ratio of second-best to best response becomes more narrowly tuned with increasing convergence (average R is 0.63, 0.59, 0.56, 0.54, 0.49). These average values, separated by best-stimulus convergence type, can be seen in figure 3.7 compared to CT data, no convergence (convergence=1), and NST data (dotted lines).

Presynaptic Inhibition Sharpens the Breadth of Tuning in Excitatory but not Inhibitory Modeled rNST Neurons

Although our modeled rNST neurons receiving stimulus-specific convergence are a good fit to the majority of the in vivo rNST data set, we considered that presynaptic inhibition might allow us to generate responses which more closely match the most narrowly tuned units recorded in vivo. We modeled presynaptic inhibition as a 50% reduction in the parameter governing the magnitude of transmitter release (PR), and found that this form of inhibition reduced the firing frequency to best-stimulus in both modeled neuron types across all four patterns and 5 levels of convergence. The average reduction relative to the baseline model was 5.3 Hz. in the excitatory model and 4.4 Hz in the inhibitory model (p<0.00002). To see this graphically, compare the open box plots representing firing frequency to the solid gray baseline responses in Figure 3.8 for all four convergence patterns (A-D) and all 5 levels of convergence (left to right within each group). Presynaptic inhibition sharpened the breadth of tuning relative to the baseline
model in the excitatory rNST model (average reduction in H was 0.05, average reduction in R was 0.07) but never did so in the inhibitory rNST model (average reduction in both H and R was 0), and this difference was highly significant (H: p<0.000001, R: p<0.0009). In Figure 3.8, this difference is reflected in the distance between the open and gray (baseline) box plots for the excitatory modeled H and R values but the nearly complete overlap between these box plots in the inhibitory model results.

*Postsynaptic Inhibition Sharpens the Breadth of Tuning in Both Excitatory and Inhibitory Modeled rNST Neurons*

Another form of inhibition which may sharpen the breadth of tuning in rNST neurons is a postsynaptic inhibitory conductance. We modeled postsynaptic inhibition as a small, tonic chloride conductance which we scaled by 0.88 in the inhibitory model neuron to reflect the smaller capacitance and size of the inhibitory rNST neurons recorded *in vitro*. Despite this scaling, postsynaptic inhibition had a significantly larger effect on the firing frequency to best stimulus of the inhibitory modeled neuron than the excitatory modeled neuron across all four patterns and 5 levels of convergence (5.6 Hz. in the excitatory model, 9.7 Hz. in the inhibitory model, p<0.0000001). This larger reduction can be seen as a larger difference between the open and solid (baseline) box plots in the frequency results presented in Figure 3.9. Postsynaptic inhibition reduced the breadth of tuning compared to the baseline model in both modeled neuron types. The average reduction in H was 0.07 in the excitatory model and 0.1 in the inhibitory model (p<0.074) and the average reduction in R was 0.09 in both the excitatory and inhibitory models (p<0.24).

*Inhibitory Synaptic Coupling Fails to Sharpen the Breadth of Tuning in Modeled rNST Neurons*

We also tested synaptic inhibition from the model inhibitory neuron to the model excitatory neuron by creating a modeled inhibitory synapse in which release was triggered by a spike in the inhibitory model cell rather than by an input rate-determined
sequence of ISIs. When we used a copy of the ST-rNST synapse model with an inhibitory reversal potential for this purpose, we found that synaptic coupling had virtually no effect on the firing rate of the excitatory model neuron (data not shown). When we removed the STSD from the synapse and slowed decay (see Table 1 for values), both changes consistent with in vitro recordings in the rNST (Grabauskas & Bradley, 1998a; 1999; 2003), synaptic coupling produced a modest decrease in the firing rate of the modeled excitatory rNST neuron (average reduction of 4.8 Hz.) but did not significantly alter its breadth of tuning (average change in H was 0, average increase in R was 0.01) compared to the baseline model. These changes can be seen in Figure 3.10 as the difference between the open and solid (baseline) box plots across all four patterns and 5 levels of convergence.

A Broadly Tuned Inhibitory Surround Sharpens the Breadth of Tuning, Particularly in the Presence of Convergence

Although a random pattern of convergence is unlikely to account for all rNST responses, it may serve as a broadly tuned “surround” input to inhibitory rNST neurons. If excitatory neurons receiving stimulus-specific “center” input received inhibition from such broadly-tuned inhibitory neurons, they would form a center-surround pattern which could in theory generate a more narrowly tuned excitatory unit.

In modeled excitatory rNST neurons receiving stimulus-specific convergence, we found that this arrangement reduced the firing frequency to best-stimulus by 3.7 Hz., reduced H by 0.04, and reduced R by 0.07 when compared to the baseline model across all 5 levels of convergence. When compared to the corresponding changes caused by synaptic inhibition by a similarly tuned surround (see Figure 3.11B-D and compare to Figure 3.10B-D), the center-surround pattern produced a significantly smaller suppression of the response to best-stimulus (p<0.0004) and a significant sharpening in H (p<0.0002) and R (p<0.000003). Center-surround inhibition had a more substantial effect on the second-best to best-stimulus ratio in modeled excitatory neurons receiving S-best and A-best input (the average reduction in R was 0.09 for both types) when
compared to its effect on N-best modeled neuron (average reduction in R was 0.04). This
difference can be seen as a wider gap between the R values in Figure 3.11 (open box
plots) and the corresponding values from the baseline model (gray filled boxes) in S-best
(3.11B) and A-best (3.11D) compared to N-best (3.11C) modeled neurons.

Discussion

The goal of this study was to fit a mathematical model to intrinsic properties of
the rNST measured in vitro and to determine whether the model could recapitulate the
modest increase in firing rate and breadth of tuning observed between the CT and rNST
in vivo (J. B. Travers & Smith, 1979). We find that a mathematical model fit to the
intrinsic properties of ST afferents and rNST neurons defines an input-output relationship
in the rNST which is an increasing, saturation function over the range of physiologically
relevant input frequencies. When firing frequencies taken from in vivo CT recordings
(Frank, 1973) are used as input to this mathematical model, we find that stimulus-specific
patterns of convergence produce modeled responses which compare favorably to in vivo
rNST recordings (J. B. Travers & Smith, 1979) but which are slightly more broadly
tuned. The average response profiles of the baseline models receiving stimulus-specific
convergence (Figure 3.12C) look very similar to the average response profiles of the in
vivo rNST data set (Figure 3.12B), particularly with respect to the orderliness of the
sideband sensitivities. Averaged across three convergence types (S,N,&A), five
convergence levels (2-10), and two model types (excitatory and inhibitory), these
modeled rNST responses have a frequency to best-stimulus of 21 Hz. (3.5 Hz. lower than
the in vivo rNST responses), an H value of 0.83 (0.12 higher than in vivo), and an R value
of 0.61 (0.1 higher than in vivo). In order to control for the different proportions of best-
stimulus types in the in vivo data set, the values reported for the in vivo data above are
averages of the average values within each best-stimulus class.

Presynaptic inhibition (50% reduction in transmitter release), postsynaptic
inhibition (a small tonic chloride conductance), and synaptic inhibition by a broadly
tuned “surround” signal (generated by random convergence of CT afferents onto a modeled inhibitory rNST neuron) sharpen the breadth of tuning in the modeled rNST responses. In affected model neurons averaged across three convergence types (S,N,&A), five convergence levels (2-10), and (where indicated) two model types, reductions in H are 0.05 (presynaptic, excitatory model), 0.075 (postsynaptic, both model types), and 0.04 (center-surround, excitatory model), and the corresponding reductions in R are 0.07, 0.09, and 0.073 (see Figure 3.13B&C). In the context of the difference in breadth of tuning (0.12 for H and 0.1 for R) between the baseline modeled rNST neurons and the target in vivo data, these seemingly small (0.04-0.09) reductions represent a significant improvement in the performance of our mathematical model and we predict that one or all of these inhibitory mechanisms are tonically active in the rNST.

Sparse Convergence Increases Firing Frequency But Not Breadth of Tuning in the rNST

Convergence of peripheral afferents onto rNST neurons has been demonstrated in the rNST in vivo between afferents with different anatomical receptive fields (Sweazey & Smith, 1987; S. P. Travers, Pfaffmann, & Norgren, 1986; Vogt & Mistretta, 1990) and in vitro between different cranial nerve tracts (Grabauskas & Bradley, 1996), and this convergence has generally been thought to underlie the increase in the breadth of tuning as gustatory information passes to the central nervous system from the periphery (for a review see (Spector & Travers, 2005)).

In our mathematical model we assume between two and ten afferents converge onto a single modeled rNST neuron, and we find that between these two extremes the breadth of tuning does not markedly increase and may in fact decrease when measured as the ratio of second-best to best-stimulus (on average +0.01 for H and -0.14 for R, see Figure 3.7). This is true only when inputs are selected from within each best-stimulus class; increasing numbers of randomly selected convergent afferents do increase the breadth of tuning as measured by both H and R (on average +0.03 for H and +0.09 for R, see Figure 3.6A).
This result is likely a consequence both the characteristics of the inputs and of the shape of the frequency in-frequency out relationship which is established by the intrinsic properties of the system (Figure 3.4A). As an example, consider an input with a frequency to best-stimulus of 20 Hz and a second-best frequency of 10 Hz (these values fall within the range of the corresponding average values in the in vivo CT data).

Assuming 2 convergent inputs, the output frequency of the excitatory model would be 11.2 Hz to the best-stimulus and 9.4 Hz to the second-best stimulus (R=0.84).

Assuming 10 convergent inputs, the same numbers are 35.6 Hz and 27.8 Hz (R=0.78).

The offset, slope, and saturation point of these curves are important determinants of the response profiles of rNST neurons, both with respect to frequency and breadth of tuning.

When we assume just one afferent input, we find that the nonlinear transformation from CT to rNST firing rates (Figure 3.4A) accounts for a substantial increase in the breadth of tuning of modeled rNST responses relative to the CT input; H increases 0.10 and R increases 0.15 when averaged across the three best-stimulus and two model types.

Responses for each best-stimulus and model type are shown individually in Figure 3.7 (compare CT data to convergence of 1). Convergence between two afferents of the same best-stimulus type results in an additional increase in H of a similar magnitude (on average +0.07) but results in a much smaller increase in R (on average +0.03). As previously mentioned, increasing the number of convergent afferents to ten results in a very minimal additional increase in H (on average +0.01) but a substantial sharpening of R (on average -0.14).

Despite Different Intrinsic Properties, Excitatory and Inhibitory rNST Neurons Have Similar Input-Output Relationships

In the present study, we find that rNST neurons have low thresholds of firing in response to applied current and have limited ability to fire action potentials in response to large currents which exceed their dynamic range. Inhibitory neurons in the rNST receive significantly smaller ST-evoked currents and have lower thresholds and smaller dynamic ranges than do excitatory neurons. Despite these differences, models fit to the average
characteristics of excitatory and inhibitory rNST neurons exhibit remarkably similar relationships between input and output frequencies (compare left and right panels in Figure 3.4A).

The intrinsic excitability of rNST neurons has been well characterized at a fixed level of positive applied current (R. M. Bradley & Sweazey, 1992; M. Wang & Bradley, 2010b), and several currents which are recruited by hyperpolarization, including A-current, H-current, and low voltage activated calcium currents (Suwabe et al., 2011; Tell & Bradley, 1994), have been shown to exist in subsets of rNST neurons. These currents are commonly expressed and A-current may have different expression patterns in the excitatory and inhibitory subpopulations of rNST neurons (M. Wang & Bradley, 2010b). Hyperpolarization-recruited conductances almost certainly influence the response of rNST neurons to inhibition, and future work incorporating A-current and H-current positive model neurons into a more complex version of our rNST network model would strengthen the results.

Short Term Synaptic Depression Sets an Appropriate Gain in the rNST

We find that STSD produces an increasing, saturating relationship between presynaptic firing frequency and evoked postsynaptic current over physiologically relevant stimulus durations in vitro (Figure 3.2), similar to what was observed over shorter stimulus durations in (M. Wang & Bradley, 2010a). When we fit a model to this relationship, it produced a similar increasing, saturating function mapping input frequencies to output frequencies (Figure 3.4A). When STSD was removed from the modeled synapse (by assuming near-instantaneous resetting of transmitter from the “unavailable” to “available” states) the resulting input-output relationship had a much steeper slope and, in the presence of even a modest degree of convergence, rapidly saturated the ability of the postsynaptic neuron to repolarize, resulting in a “crash” in the output firing frequency at input frequencies in the physiological range (Figure 3.4B). This suggests that the function of STSD in gustatory afferents the rNST is to maintain the
slope of the input-output relationship such that it falls within the dynamic range of the rNST neurons.

*Stimulus-specific patterns of convergence produce modeled rNST responses comparable to in vivo recordings*

Convergence of *in vivo* CT recordings selected according to best-stimulus type produced modeled responses were remarkably similar to *in vivo* rNST data both in terms of their firing frequency to best-stimulus and their breadth of tuning (Figure 3.6B,C,D; Figure 3.12B,C). This stimulus-specific convergence pattern parallels that seen in the olfactory system, in which odorant receptor cells of the same type project to specific glomeruli (Hildebrand and Shepherd, 1997) and the auditory system, in which afferents converge (Cao and Oertel 2010) in an orderly fashion to create a tonotopic map in the cochlear nuclei (find the original source, cite one of many modern sources?).

Although the responses of the model assuming stimulus-specific convergence were a reasonably good fit to the *in vivo* rNST data, the model failed to account for any rNST response greater than 40 Hz. (Figure 3.6B,C,D). This is a consequence of the input-output relationship produced by fitting both the modeled synapse and the modeled rNST neurons to the average characteristics of the excitatory and inhibitory rNST neurons recorded *in vitro* (Figure 3.4). It is possible that a modeling approach which reflected the heterogeneity of the *in vitro* data (see error bars in Figures 3.2 and 3.3) would produce a heterogeneity of input-output relationships which would allow us to capture the behavior of these fast-firing rNST neurons. Alternately, it is possible that rNST neurons with responses to best-stimulus of greater than 50 Hz. (n=1 N-best and 5 A-best, see Figure 3.5) represent a subtype which is produced by the selective combination of afferents and postsynaptic neurons capable of evoking and sustaining such a response.

In contrast, input rates selected at random produced inappropriately broadly tuned responses (Figure 3.6A, Figure 3.12B,C) with a disproportionate incidence of salt-best modeled neurons. This is likely the result of the orderliness of the sideband sensitivities
in the *in vivo* chorda tympani data used as input to the model (Figure 3.5A, Figure 3.12A); although salt-best afferents are not disproportionately represented, salt is most likely to be the second-best stimulus in both sweet-best and acid-best afferents (J. B. Travers & Smith, 1979).

*Pre- and Postsynaptic Inhibition Sharpens the Breadth of Tuning in Modeled rNST Neurons*

The baseline rNST model failed to account for some more narrowly tuned *in vivo* responses, in particular in the salt-best and acid-best groups. Tonic inhibition has been shown to sharpen the breadth of tuning in the rNST *in vivo* (Smith & Li, 1998), and so we considered the possibility that adding inhibition to our models would result in a sharpening of the breadth of tuning and therefore a better fit to the *in vivo* data. We tested two types of stimulus-independent inhibition: a presynaptic suppression of release and a small postsynaptic chloride current.

Presynaptic inhibition reduced the firing frequencies to best stimulus of both excitatory and inhibitory rNST neuron models by a similar amount, but was only effective at sharpening the breadth of tuning of the excitatory rNST neuron model (Figure 3.8, red bars in Figure 3.13). It is possible that presynaptic inhibition is sufficient to reduce the response rate to low frequencies in the excitatory modeled neuron (which has a larger ST-evoked postsynaptic current but also a higher threshold), while in the inhibitory modeled neuron the reduced synaptic current still exceeds its lower threshold to fire action potentials. This differential effect could explain why firing frequencies in response to lower-frequency sideband sensitivities are preferentially reduced (thereby sharpening the breadth of tuning) only in the excitatory neuron model.

Activation of a small postsynaptic chloride conductance was effective at sharpening the breadth of tuning in both modeled neuron types (Figure 3.9, green bars in Figure 3.13). Since it has been reported that postsynaptic inhibition in the presence of STSD produces a gain change (Rothman, Cathala, Steuber, & Silver, 2009) and since a purely divisive gain change would not alter the breadth of tuning as measured by
proportion-based measures, this result was somewhat surprising. However, the gain change demonstrated in Rothman et al. was accompanied by a substantial subtractive shift of the input-output relationship (Rothman et al., 2009), and this subtractive shift could account for the sharpening of the breadth of tuning in our modeled rNST responses.

**Synaptic Inhibition Sharpens the Breadth of Tuning When Inhibitory Neurons are Broadly Tuned**

We found that synaptic inhibition of the excitatory neuron model by the inhibitory neuron model had no effect on the breadth of tuning when both neuron types received input of the same best-stimulus type (Figure 3.10, purple bars in Figure 3.13), but did result in a modest sharpening of the breadth of tuning when the excitatory rNST neuron model received a stimulus-specific “center” signal and the inhibitory model received a randomly selected “surround” signal (Figure 3.11, orange bars in Figure 3.13). This produced a better fit to the in vivo data in the excitatory rNST neuron model, particularly with respect to R in S-best and A-best modeled rNST neurons. Since the modeled inhibitory neurons receiving randomly selected input are likely to be broadly tuned N-best (Figure 3.6A, Figure 3.12 C, far right), and since the second-best stimulus in both S-best and A-best modeled rNST neurons is likely to be salt (Figure 3.12 C), it follows that inhibition from a broadly tuned N-best “surround” would preferentially sharpen the breadth of tuning, as measured by R, in S-best and A-best modeled rNST neurons. If this center-surround arrangement is hard-wired as a sharpening mechanism in the microcircuitry of the rNST, the model predicts that inhibitory neurons would be more broadly tuned and more likely to be salt-best than neighboring excitatory rNST neurons.

This center-surround strategy of inhibition is thought to occur in the olfactory system, in which a broadly tuned inhibitory signal presynaptically inhibits odorant-specific input (Olsen, S. R., Bhandawat, V., & Wilson, 2010) and also in the auditory system, in which local glyceinergic interneurons provide broadly tuned inhibition to projection neurons receiving narrowly tuned tonotopic input (Campagnola and Manis, 2014)
Predictions

Based on the results of this study, we predict that convergence in the rNST is largely between afferents of the same best-stimulus type, although it is possible that a subset of rNST neurons may receive input that is less segregated by best-stimulus type. We find that virtually every tested level of convergence contributes to the goodness of fit to the in vivo rNST data under some combination of stimulus-specific convergence and inhibition type. This does not necessarily predict heterogeneity in the degree of convergence in the rNST, but does suggest that it is plausible. We think that presynaptic inhibition, if present in the rNST, would sharpen the breadth of tuning of excitatory but not inhibitory rNST neurons, while tonic postsynaptic inhibition would sharpen the breadth of tuning in both rNST neuron types. Furthermore, we predict that a center-surround inhibition strategy which assumes random convergence onto the inhibitory neurons would effectively sharpen the breadth of tuning in some rNST neurons, but it would also require that participating inhibitory neurons be broadly tuned and most often salt-best.
Figure 3.1
Structure of the Mathematical Model

A diagram of the structure of the mathematical model
Figure 3.2
ST-evoked currents in rNST neurons

A. Venus -  

<table>
<thead>
<tr>
<th>5 Hz</th>
<th>30 Hz</th>
<th>50 Hz</th>
</tr>
</thead>
</table>

Venus +  

B.  

<table>
<thead>
<tr>
<th>Response Magnitude (nA*ms)</th>
</tr>
</thead>
</table>

Figure 3.2

Example traces in (A) show that ST-evoked currents in both Venus-negative (left) and Venus-positive (right) rNST neurons display frequency-dependent STSD. Examples shown are representative of the average magnitude for each neuron type.

The average response magnitude (B) for the population of Venus-negative (n=21) and Venus-positive (n=21) responses is an increasing, saturating function. Response magnitude is measured in units of nA*ms and corresponds to the area under the curve during the 500 ms stimulus period. Blue and green symbols indicate population averages and red and pink symbols indicate the performance of the excitatory and inhibitory rNST neuron models, respectively when subjected to an identical protocol. Error bars are standard error of the mean.
Figure 3.3

Example traces in (A) show the responses of Venus-negative (left) and Venus-positive (right) rNST neurons to a current-clamp injection protocol. Selected current values illustrate typical responses of these neuron types to a subset of applied current steps.

Averaged values in (B) show the response of the population to all applied current steps, error bars indicate the standard error of the mean. Note that the x-axis for the I-V portion of the curve is applied current while the x-axis for the frequency response portion of the curve is applied current above threshold. This is necessary to minimize the distortion of averaging on the shape of the relationship between applied current and firing frequency in the two cell types.

Measured values for threshold, break point, and maximum firing frequency are shown as box plots (B, inset). The superimposed red and pink bars show the values of the corresponding parameters in the behavior of the excitatory and inhibitory rNST model neurons when put through a protocol identical to the patch-clamp protocol.
Figure 3.3
Intrinsic Excitability of rNST Neurons

A. Venus -  Venus +  B.

-0.1 nA
0.01 nA
0.05 nA
0.1 nA
0.19 nA

Firing Frequency (Hz)
Voltage (mV)

-150 -100 -50 0 50 100

-0.2 -0.1 0 0.1 0.2 0.3

Threshold (nA) Break Point (nA) Maximum Firing Frequency (Hz)

VENUS-
Data
Excitatory
Model
VENUS+
Data
Inhibitory
Model

p<0.005

*
Figure 3.4

(A) The relationship between input (x-axis) and output (y-axis) frequencies in the modeled rNST neurons is an increasing, saturating function. Convergence produces a progressive increase in the slope between in input and output frequencies, and furthermore these relationships are strikingly similar in the excitatory (left) and inhibitory (right) modeled rNST neurons.

(B) The same plots as in (A), but with the effect of STSD eliminated by assuming instantaneous resetting of available transmitter.
Figure 3.4
Convergence in the rNST

A. Excitatory Model

B. Excitatory Model, no STSD
Figure 3.5

In vivo data from CT ((Frank, 1973)) and rNST ((J. B. Travers & Smith, 1979)) are grouped by best-stimulus type (symbols) and their frequency to best-stimulus (x-axis) is plotted against the entropy measure of their breadth of tuning (y-axis). The rightward and upward shift allow the slight increase in frequency and increase in the breadth of tuning reported in the original work to be visualized.
Figure 3.6

(A) The responses of the excitatory (left) and inhibitory (right) modeled rNST neurons to random convergence of afferents drawn from the entire population of in vivo CT responses. The box plots show (left to right) the firing frequency to best stimulus (y-axis is 0-120 Hz), the entropy measure of breadth of tuning (y-axis is 0-1) and the ratio of second-best to best response (y-axis is 0-1). Plotted left to right within these groupings is the in vivo CT data used as input to the model (white bar), the comparable in vivo rNST data (dark gray bar), and the model results assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs.

(B-D) The responses of the excitatory (left) and inhibitory (right) modeled rNST neurons to random convergence of afferents drawn from the sucrose-best (B), sodium-best (C), and acid-best (D) populations of in vivo CT responses. All in vivo data presented are the same best-stimulus type as the modeled responses, but otherwise the panels are the same format as the panels described in (A).
Figure 3.6
Baseline Model

A. Random CT Data

B. S-Best CT Data

C. N-Best CT Data

D. A-Best CT Data
Figure 3.7

The Effect of Stimulus-Specific Convergence on H and R

Figure 3.7

The H and R metrics of breadth of tuning for excitatory (open circles) and inhibitory (open triangles) modeled rNST neurons receiving S-best (red), N-best (blue) or A-best (green) input are plotted against convergence number (1, 2, 4, 6, 8, and 10) and can be compared to CT data (solid circles) and rNST data (dotted lines). Each point represents the average of all responses in that best-stimulus class (CT, NST data) or the average of 25 model runs.
Figure 3.8

Modeled rNST responses to convergence of random (A), S-best (B), N-best (C), and A-best (D) in vivo CT responses in the presence of presynaptic inhibition. Gray bars are control responses from figure 5; overlying bars are modeled responses with presynaptic inhibition (50% reduction in release).

Within each section, left panels are the responses of the excitatory rNST neuron model, and right panels are the responses of the inhibitory rNST neuron model. The box plots show (left to right) the firing frequency to best stimulus (y-axis is 0-120 Hz), the entropy measure of breadth of tuning (y-axis is 0-1) and the ratio of second-best to best response (y-axis is 0-1). Plotted left to right within these groupings is the in vivo CT data used as input to the model (white bar), the comparable in vivo rNST data (dark gray or colored bar), and the model results assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs.
Figure 3.8
Presynaptic Inhibition

A. Random CT Data

B. S-Best CT Data

C. N-Best CT Data

D. A-Best CT Data
Figure 3.9

Modeled rNST responses to convergence of random (A), S-best (B), N-best (C), and A-best (D) *in vivo* CT responses in the presence of postsynaptic inhibition. Gray bars are control responses from figure 5; overlying bars are modeled responses with a small postsynaptic chloride conductance.

Within each section, left panels are the responses of the excitatory rNST neuron model, and right panels are the responses of the inhibitory rNST neuron model. The box plots show (left to right) the firing frequency to best stimulus (y-axis is 0-120 Hz), the entropy measure of breadth of tuning (y-axis is 0-1) and the ratio of second-best to best response (y-axis is 0-1). Plotted left to right within these groupings is the *in vivo* CT data used as input to the model (white bar), the comparable *in vivo* rNST data (dark gray or colored bar), and the model results assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs.
Figure 3.9
Postsynaptic Inhibition

A. Random CT Data

B. S-Best CT Data

C. N-Best CT Data

D. A-Best CT Data
Figure 3.10

Modeled rNST responses to convergence of random (A), S-best (B), N-best (C), and A-best (D) in vivo CT responses in the presence of a similarly tuned inhibitory surround. Gray bars are control responses from figure 5; overlying bars are responses of the excitatory rNST neuron model receiving synaptic inhibition from the inhibitory rNST neuron model. Both models are receiving the same type of CT data as input.

The box plots show (left to right) the firing frequency to best stimulus (y-axis is 0-120 Hz), the entropy measure of breadth of tuning (y-axis is 0-1) and the ratio of second-best to best response (y-axis is 0-1). Plotted left to right within these groupings is the in vivo CT data used as input to the model (white bar), the comparable in vivo rNST data (dark gray or colored bar), and the model results assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs.
Figure 3.10
Synaptic Inhibition

A. Random

Excitatory Model

Scale = 0-1 (breadth of tuning measures)
Scale = 0-120 Hz (frequency measure)

B. S-Best

C. N-Best

Excitatory Model

D. A-Best

Excitatory Model

Scale = 0-1 (breadth of tuning measures)
Scale = 0-120 Hz (frequency measure)

Firing Frequency
Breadth of Tuning (H)
Breadth of Tuning (R)
Figure 3.11

Modeled rNST responses to convergence of random (A), S-best (B), N-best (C), and A-best (D) in vivo CT responses in the presence of a broadly tuned inhibitory surround. Gray bars are control responses from figure 5; overlying bars are responses of the excitatory rNST neuron model receiving synaptic inhibition from the inhibitory rNST neuron model. The inhibitory neuron model receives convergent input from randomly selected in vivo CT responses in every panel.

The box plots show (left to right) the firing frequency to best stimulus (y-axis is 0-120 Hz), the entropy measure of breadth of tuning (y-axis is 0-1) and the ratio of second-best to best response (y-axis is 0-1). Plotted left to right within these groupings is the in vivo CT data used as input to the model (white bar), the comparable in vivo rNST data (dark gray or colored bar), and the model results assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs.
Figure 3.11
Synaptic Inhibition with a Broadly Tuned Surround

A. Random

B. S-Best

C. N-Best

D. A-Best

Baseline Model Results (Fig. 6)
Model I Results with 2, 4, 6, 8, 10 Afferents

Excitatory Model

Scale = 0-1 (breadth of tuning measures)
Scale = 0-120 Hz (frequency measure)

Firing Frequency
Breadth of Tuning (H)
Breadth of Tuning (R)
The mean response profiles for all *in vivo* data and model outputs reported in the present study, including CT data (A), rNST data (B), baseline model results (C), modeled presynaptic inhibition (D), modeled postsynaptic inhibition (E), synaptic inhibition (F), and synaptic inhibition with a broadly tuned inhibitory surround (G). Responses are segregated by their best-stimulus (*in vivo* data) or by the type of convergence which produced them (model results). All model results are averages of 25 runs at each of five levels of convergence (2, 4, 6, 8, and 10) for a total of 125 modeled responses, and error bars are standard error of the mean.
Figure 3.12
Mean Response Profiles for In Vivo Data and Modeled rNST Responses

A. S & N & A & Q
S-best CT Data & N-best CT Data & A-best CT Data & Q-best CT Data

B. S & N & A & Q
S-best NST Data & N-best NST Data & A-best NST Data & Q-best NST Data

C. S & N & A & Q
Baseline Excitatory Model

D. S & N & A & Q
Presynaptic Inhibition of Excitatory Model

E. S & N & A & Q
Postsynaptic Inhibition of Excitatory Model

F. S & N & A & Q
Synaptic Inhibition of Excitatory Model

G. S & N & A & Q
Synaptic Inhibition of Excitatory Model by a Broadly Tuned Surround
Figure 3.13

The mean values for frequency to best stimulus (A), entropy (H), and ratio of second-best to best-stimulus (C) for modeled results, including the baseline model (blue), presynaptic inhibition (red), postsynaptic inhibition (green), synaptic inhibition (purple), and synaptic inhibition with a broadly tuned inhibitory surround (orange). Each bar is an average of 25 model runs assuming (left to right) 2, 4, 6, 8, and 10 convergent afferent inputs drawn from S-best, N-best, or A-best populations. The corresponding values for the in vivo rNST data are plotted for comparison as the top edge of the gray shaded box for each metric and best-stimulus class.
Figure 3.13
Average Values for Frequency and Breadth of Tuning of Modeled NST Neurons

A. Frequency to Best-Stimulus
   Scale 0-40 Hz.

B. Breadth of Tuning (H)
   Scale 0-1

C. Breadth of Tuning (R)
   Scale 0-1

Baseline  Presynaptic  Postsynaptic  Synaptic  Center-surround

2, 4, 6, 8, 10
Convergence Number
<table>
<thead>
<tr>
<th>ST Synapse Model Parameters</th>
<th>Inhibitory Model (empty cells indicate identical values were used)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excitatory Model</strong></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td>R</td>
<td>500</td>
</tr>
<tr>
<td>PR</td>
<td>0.118</td>
</tr>
<tr>
<td>Gsyn</td>
<td>0.1658 uS</td>
</tr>
<tr>
<td>Esyn</td>
<td>0 mV</td>
</tr>
<tr>
<td><strong>Inhibitory Model</strong></td>
<td></td>
</tr>
<tr>
<td><strong>rNST Neuron Model Parameters</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Excitatory Model</strong></td>
<td></td>
</tr>
<tr>
<td>Cm</td>
<td>0.0187</td>
</tr>
<tr>
<td>Gna</td>
<td>0.24 uS</td>
</tr>
<tr>
<td>Gk</td>
<td>0.011 uS</td>
</tr>
<tr>
<td>Gks</td>
<td>0.003 uS</td>
</tr>
<tr>
<td>Gleak</td>
<td>0.0018 uS</td>
</tr>
<tr>
<td>Gel (when present)</td>
<td>0.001 uS</td>
</tr>
<tr>
<td>Ena</td>
<td>50 mV</td>
</tr>
<tr>
<td>EK</td>
<td>-90 mV</td>
</tr>
<tr>
<td>ELeak</td>
<td>-59.5 mV</td>
</tr>
<tr>
<td>Ecl</td>
<td>-70 mV</td>
</tr>
<tr>
<td><strong>Gating Parameters with the Same Values in Both Models</strong></td>
<td></td>
</tr>
<tr>
<td>$\phi_{m_{\text{min}}}$</td>
<td>-38</td>
</tr>
<tr>
<td>$O_{m_{\text{min}}}$</td>
<td>5</td>
</tr>
<tr>
<td>$\phi_{h_{\text{min}}}$</td>
<td>-50</td>
</tr>
<tr>
<td>$O_{h_{\text{min}}}$</td>
<td>3</td>
</tr>
<tr>
<td>$\phi_{n_{\text{min}}}$</td>
<td>-40</td>
</tr>
<tr>
<td>$O_{n_{\text{min}}}$</td>
<td>5</td>
</tr>
<tr>
<td>$\phi_{m_{\text{ns}}}$</td>
<td>-40</td>
</tr>
<tr>
<td>$O_{m_{\text{min}}}$</td>
<td>5</td>
</tr>
<tr>
<td>$\phi_{h_{\text{ns}}}$</td>
<td>-20</td>
</tr>
<tr>
<td>$O_{h_{\text{ns}}}$</td>
<td>-10</td>
</tr>
<tr>
<td>$\phi_{h_{\text{Tn}}}$</td>
<td>-60</td>
</tr>
<tr>
<td>$O_{h_{\text{Tn}}}$</td>
<td>3</td>
</tr>
<tr>
<td>$\phi_{h_{\text{Tns}}}$</td>
<td>-45</td>
</tr>
<tr>
<td>$\phi_{a_{\text{ns}}}$</td>
<td>-3</td>
</tr>
<tr>
<td>$\phi_{a_{\text{Tns}}}$</td>
<td>-50</td>
</tr>
</tbody>
</table>

Table 1. Model Parameter Values
Chapter 4: Discussion

The rostral nucleus of the solitary tract (rNST) receives gustatory afferent input from the periphery and relays it to other central structures, which may be broadly subdivided into an ascending pathway via the parabrachial nucleus and a local medullary pathway via the reticular formation and caudal NST. At this first central node in the gustatory system, the signal is modulated by input from systems tasked with gustatory perception, nutritional and body weight homeostasis, and digestive and satiety function, as well as by circulating hormones and peptides.

The mechanisms and consequences of modulation in this system are central to an understanding of its normal function, and modulation by the opioid receptor agonist met-enkephalin has been shown to dramatically suppress the responses of a subset of gustatory rNST neurons in vivo (Li, Davis, & Smith, 2003). There are three major classes of opioid receptors: delta, mu, and kappa, and the activation of these receptors may give rise to different effects which generally act to inhibit neuronal responsiveness. The in vivo effect on the gustatory response was ascribed to the activation of postsynaptic δ-opioid receptors on the strength of an in vitro patch clamp paper (Zhu, Cho, & Li, 2009), but the effect of µ-opioid receptor (MOR) activation in the rNST was not studied. Our lab found that infusing the MOR agonist DAMGO into the rNST in vivo altered taste-elicited oromotor patterns associated with ingestion and rejection, demonstrating a functional role for MORs in rNST modulation (Kinzelger & Travers, 2011). The work presented in Chapter 2 shows that DAMGO presynaptically suppresses solitary tract (ST)-evoked responses in both excitatory and inhibitory rNST neurons in an in vitro patch clamp preparation. A previous paper found that DOR activation also suppressed ST-evoked responses, but did so via a postsynaptic mechanism (Zhu et al., 2009). These
electrophysiological findings are consistent with immunohistochemical results which suggest that MORs are predominantly presynaptically localized while DORs are primarily postsynaptically localized in the rNST (Li et al., 2003). It is likely, then, that the application of met-enkephalin would doubly suppress ST input to rNST neurons; first presynaptically suppressing release via MOR activation and then further reducing the current amplitude via postsynaptic DOR activation. Although both of these effects presumably blunt the transmission of gustatory information in the rNST, the details of how they act, individually or in combination, to shape rNST responses to natural gustatory input remain unclear.

One intriguing possibility which remains to be explored is the potential for opioid modulation to activate a postsynaptic inhibitory current in rNST neurons. Given the localization of MORs to ST terminals, it is not surprising that we did not see this effect frequently in the experiments presented in Chapter 2. However, by holding at -70 mV we were almost certainly minimizing the impact of a small inhibitory current with a reversal potential near the holding potential. This design choice is shared by a study demonstrating the effect of met-enkephalin and DOR agonists in the rNST (Zhu et al., 2009), and so it is possible that postsynaptic inhibition has been overlooked and underestimated as a mechanism for opioid modulation in the rNST. Because even small postsynaptic inhibitory conductances can have a profound impact on the input-output relationship in the rNST (as demonstrated in the modeling studies in Chapter 3), the possibility of this form of modulation is a potentially important open avenue for further study.

Studies of the fundamental biophysical properties of the rNST and associated modulatory mechanisms are an important step to understanding how these mechanisms shape rNST function. It is sometimes difficult, however, to make a connection between a property observed in a reduced preparation and the function of the full-scale system. An example of this in the rNST is short term synaptic depression (STSD), first observed in vivo as a change in the probability of a response to the second of two closely spaced electrical stimulations of a peripheral input (Hallock & Di Lorenzo, 2006; Lemon & Di
Lorenzo, 2002; Rosen & Di Lorenzo, 2009). Although it is possible that polysynaptic inhibition contributes to this effect, its persistence in vitro in the presence of blockers of inhibitory synaptic transmission (Wang & Bradley, 2010) makes it unlikely to account for it in its entirety. The effect of STSD on ST-evoked EPSCs is significant, and occurs well within the input frequency range known to be physiologically relevant in the rNST (see Chapter 3). It has been speculated that STSD results in a frequency-dependent filter in the rNST (Wang & Bradley, 2010), preventing the passage of high frequency ST inputs. If a typical ST-evoked response is minimally sufficient to evoke a postsynaptic action potential this is likely to be true, but if a typical ST-evoked response is 100x bigger than threshold, it is likely to be robust to even a large reduction due to STSD. In fact, we found that in the mathematical model presented in Chapter 3, which was fit to measured characteristics of ST-evoked responses as well as to measures of postsynaptic excitability, STSD produced a saturation rather than a drop in the input-output relationship. This effect produces a type of frequency-dependent filter, but rather than preventing transmission of high frequency input, it facilitated this transmission by keeping the input scaled within the dynamic range of our modeled rNST neurons. When we removed STSD from the model, we found that the synaptic input rapidly exceeded the ability of the modeled neurons to repolarize, resulting in a reduction in the output frequency in response to high input frequencies. That the removal of STSD from the model caused the phenomena that it was itself predicted to cause illustrates the utility of a computational approach to exploring the contributions of various mechanisms to rNST function.

In our modeling approach, we focused on capturing phenomena like threshold, firing rate, and STSD rather than making precise measurements of the contributions of individual currents of subcellular mechanisms. This allowed us to maintain a (relatively) simple model, while still capturing behavior relevant to the fundamental function of the system: transforming input rates from peripheral afferents to output rates. We explored the effect of, and interactions between, STSD and convergence of peripheral afferents using input-output relationships across the range of physiologically relevant input
frequencies. We also ran a series of experiments in which we used randomly selected rates derived from \textit{in vivo} chorda tympani recordings (Frank, 1973) as input to the model. Using this approach, we were able to model rNST responses to convergent inputs with physiologically realistic variation. The existence of another, parallel \textit{in vivo} data set from the rNST (J. B. Travers & Smith, 1979) allowed us to directly compare these modeled responses to the output of the real system, both with respect to firing rates and breadth of tuning.

The breadth of tuning refers to the degree to which a gustatory afferent or neuron responds selectively to one or more broadly to many gustatory stimuli applied to the tongue. Breadth of tuning modestly increases in the rNST compared to peripheral afferents, and it has been hypothesized that this (and a corresponding modest increase in firing rate) is a result of convergence of peripheral afferents onto rNST neurons (J. B. Travers & Smith, 1979). Our modeling results clearly demonstrated increasing in firing frequency with increasing numbers of convergent inputs to a modeled neuron, but the increase in breadth of tuning had a more interesting relationship. Breadth of tuning, as measured by entropy (H) (J. B. Travers & Smith, 1979), increased as a function of simply passing through the rNST. Convergence between two afferents of the same best-stimulus type resulted in an additional increase in the breadth of tuning of approximately the same magnitude, but the breadth of tuning did not increase beyond this point with increasing numbers of afferents (up to ten). This was not true of modeled rNST neurons which received random convergence from afferents with multiple best-stimulus types; the H of these modeled neurons continued to increase with increasing convergence. These results demonstrate that convergence does increase the breadth of tuning, but that this effect does not scale with the amount of convergence unless the afferents are of different best-stimulus types. The ratio of the second-best to best-stimulus (R) also increased as a function of passing through the ST-rNST synapse and with convergence of an additional afferent of the same best-stimulus type, but then decreased with additional afferents up to ten. By ten convergent afferents, the R of the modeled rNST response was smaller than
the R of the comparable *in vivo* rNST data and was very nearly as narrowly tuned as the
CT input to the model.

These modeled results have some bearing on the long-standing discussion of
convergence and breadth of tuning in the rNST. First, the model provides a quantitative
estimate of how much the breadth of tuning might increase assuming different numbers
and patterns of convergent afferents. In general, this increase is in excess of the increase
observed in the *in vivo* data sets (Frank, 1973; J. B. Travers & Smith, 1979). This
suggests the need for additional, tonically active mechanisms for sharpening the breadth
of tuning in the rNST model to match the *in vivo* results. Tonic inhibition is a good
candidate for this mechanism, having been shown already to sharpen the breadth of
tuning of rNST neurons *in vivo* (Smith & Li, 1998). Second, our model predicts that a
significant portion of the increase of the breadth of tuning in the rNST compared to
peripheral afferents is a result of simply passing through the ST-rNST synapse. That is,
intrinsic properties of the system contribute to an increase in the breadth of tuning which
was not previously appreciated and quantified. Third, our model predicts that
convergence between afferents of the same best-stimulus type can contribute to the
sharpening of the breadth of tuning in rNST neurons, when the breadth of tuning is
measured as the ratio of second-best to best-stimulus. That increasing convergence may
contribute to maintaining the specificity of the gustatory signal is somewhat
counterintuitive, but may be a novel explanation for how the system overcomes the
intrinsic properties which tend to increase the breadth of tuning.

The above predictions assume that convergence between peripheral afferents in
the rNST is primarily within best-stimulus groups. This is certainly not always the case,
but in our modeled neurons it was impossible to recapitulate a significant population of
more narrowly tuned rNST *in vivo* responses by assuming that convergence is entirely
random. Entirely random convergence produced modeled rNST neurons which were
usually inappropriately broadly tuned and salt-best, likely as a result of the prevalence of
salt responses in the CT data: if salt was not the best-stimulus it was often the second-best
stimulus. It is possible that entirely random convergence has a role in the rNST, possibly
as a broadly tuned inhibitory “surround” signal that functions to sharpen the breadth of tuning, but our model predicts that it is unlikely to be the primary “rule” by which gustatory afferents converge onto central neurons. Both entirely random and entirely specific convergence represent the extremes on a continuum, and future work with our mathematical model could explore the middle of this continuum by allowing different proportions of convergent afferents to be selected either entirely at random or from within the same best-stimulus group. Presupposing that convergence within best-stimulus groups represents a desirable “signal”, this work could be interpreted as quantifying the degree to which the system is robust to errors in wiring, a desirable trait in a system with such rapid turnover in receptor cells and the associated peripheral plasticity.

It is possible that random convergence does not represent an error, but rather is a feature of the system with a role in its normal functioning. Our modeling work demonstrates that random convergence of CT afferents onto an inhibitory modeled rNST neuron can produce an effective center-surround sharpening mechanism, in which a stimulus-specific “center” signal is inhibited by a broadly responsive “surround.” If present, this circuitry would have to be a hard-wired part of the rNST and would most significantly shape the rNST response to mixtures of tastants.

Inhibition in the rNST is generally thought to be a target for modulation, and tonic inhibition has been demonstrated in the rNST in vivo, where it was shown to sharpen the breadth of tuning of affected gustatory neurons (Smith & Li, 1998). These results could be consistent with an inhibitory circuit receiving gustatory input, such as the center-surround mechanism previously discussed, but since the presence of tonic inhibition has also been demonstrated in vitro in the absence of any stimulated afferent inputs (Liu, Behbehani, & Smith, 1993), it is likely that tonic inhibition in the rNST is the consequence of a stimulus-independent, tonically active local inhibitory network. If a tonically active inhibitory signal were to reduce a rNST neuron’s response to the application of all tastants by an equal amount, this change would have a proportionately larger effect on the smaller, “sideband” responses than on the response to the neuron’s
best-stimulus, and would therefore sharpen the breadth of tuning as was demonstrated *in vivo* (Smith & Li, 1998). Even if the specific mechanism of tonic inhibition in the rNST were known, it would be difficult to show that this mechanism was responsible for this behavior, since other features of the system, such as STSD, may interact with inhibition to produce different effects on the output of the system than inhibition alone (Rothman, Cathala, Steuber, & Silver, 2009). We addressed these interactions using our mathematical model of the rNST, and found that both presynaptic and postsynaptic inhibition (modeled as a constant chloride conductance similar to that which might result from activation of extrasynaptic GABA receptors by transmitter spillover) produced a sharpening of the breadth of tuning of the modeled responses. This brought the breadth of tuning of the modeled rNST responses more closely in line with the breadth of tuning of the *in vivo* data, indicating that either of these mechanisms may account for the tonic inhibition observed *in vivo*. The notable exception to this behavior was presynaptic inhibition in the inhibitory rNST neuron model, which did not sharpen the breadth of tuning as it did in the excitatory modeled rNST neuron. This illustrates the importance of considering intrinsic properties such as those which differentiate between excitatory and inhibitory modeled rNST neurons when assessing the effect of inhibition or modulation on the input-output relationship of a neural system. In conclusion, the transfer of the afferent gustatory signal to second order neurons is significantly shaped by the subtle interplay between intrinsic properties of ST afferents and rNST neurons, convergence, inhibition, and other modulatory mechanisms.
References


Wang, M., & Bradley, R. M. (2010a). Synaptic characteristics of rostral nucleus of the solitary tract neurons with input from the chorda tympani and glossopharyngeal nerves. Brain research, 1328, 71–78. doi:10.1016/j.brainres.2010.03.003


