DENDRITIC PROPERTIES OF INHIBITORY THALAMIC NEURONS: IMPLICATIONS IN SUB-CORTICAL SENSORY PROCESSING

BY

SHANE ROBERT CRANDALL

DISSERTATION

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Doctoral Committee:

Professor Charles Leroy Cox III, Chair, Director of Research
Associate Professor Claudio F. Grosman
Assistant Professor Pritesh K. Pandya
Assistant Professor Daniel Adolfo Llano
Assistant Professor Hee Jung Chung
This dissertation is focused on understanding the cellular mechanisms underlying thalamocortical network activities. Specifically, I am interested in how inhibitory neurons of the thalamus process information received from other neurons before passing it along to other network targets. Like many neurons in the central nervous system, inhibitory neurons of the thalamus receive thousands of synaptic inputs from other neurons, most of which contact their treelike extensions called dendrites. When these inputs are activated they create electrical signals that travel across the dendrites. If the signals are strong enough, the neuron will generate action potentials, thereby communicating the information to other neurons. The process of turning input into output is often referred to as “synaptic integration” and is a fundamental process performed by all neurons. I believe understanding how information integration occurs within the dendrites of inhibitory thalamic neurons, as it relates to their organization within the network, will provide valuable insight as to the function of inhibition during thalamocortical activities. The importance of this work lies in the fact that brain processes such as sensory perception, behavioral arousal, attention, and certain pathophysiological conditions such as epilepsy result from the coordinated activities of inhibitory and excitatory neurons in the thalamocortical circuit. In Chapter 1, I provide the reader with a comprehensive review of the research literature examining inhibitory thalamic neurons. The information presented in this chapter provides a detailed background of my completed studies (Chapter 2, 3, and 4).

My initial study (Chapter 2) demonstrates that in thalamic reticular neurons, voltage-gated T-type calcium channels, located in distal dendrites, function to amplify excitatory afferent inputs. This powerful dendritic property ensures integration of distal input at the somatic level by
compensating for any attenuation that would otherwise normally occur due to passive membrane properties. Given the unique voltage-sensitivity of the T-type calcium channel, our data suggests that the degree in which synaptic input would be “boosted” would strictly depends on the voltage-state of the somatodendritic axis. Moreover, if we consider the unique structural organization of the thalamic reticular nucleus, we hypothesize that such dendritic properties could facilitate intra- and cross-modal sensory integration at the level of the thalamus. This study is published in the Journal of Neuroscience.

In my second study (Chapter 3), I show that the presynaptic dendrites of thalamic interneurons operate as independent input-output devices. This unique property allows the dendrites of thalamic interneurons to tightly regulate fast monosynaptic excitation in thalamocortical relay neurons. Given dendritic terminals operate independently of the axon and presumably each other, these results suggest that thalamic interneurons can function as multiplexing integrators. This study is published in the Journal of Neuroscience.

In my last study (Chapter 4), I reveal that the dendrites of thalamic interneurons form two types of dendrodendritic synapses in the visual thalamus. These inhibitory dendrodendritic synapses targeted the same postsynaptic neurons and are either mediated by ionotropic or a combination of ionotropic and metabotropic glutamate receptors. Two types of dendrodendritic synapses suggest that retinogeniculate input can drive different inhibitory activity in the same relay neuron, through two distinct feedforward inhibitory pathways. Inhibitory output from these two terminals would likely shape how thalamocortical relay neurons respond to visual stimuli and communicate information to the neocortex.
This work is dedicated to my loving fiancée and best friend Erin
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University of Illinois
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# Table of Contents

## Chapter 1: Introduction
1.0.0 How inhibition shapes neural network activity ......................... 1
1.1.0 Sensory processing and the thalamocortical circuit ...................... 2
1.2.0 The thalamus ........................................................................ 3
1.3.0 Thalamic relay neurons .......................................................... 4
1.4.0 Local thalamic interneurons .................................................... 8
1.5.0 The thalamic reticular nucleus ................................................. 17
1.6.0 Closing remarks ..................................................................... 25
1.7.0 Figures ................................................................................. 27
1.8.0 References .......................................................................... 31

## Chapter 2: Low-threshold Ca\(^2\+) current (I\(_T\)) amplifies distal dendritic signaling in thalamic reticular neurons
2.0.0 Abstract ............................................................................. 45
2.1.0 Introduction ......................................................................... 46
2.2.0 Methods and materials .......................................................... 48
2.3.0 Results ............................................................................... 51
2.4.0 Discussion .......................................................................... 60
2.5.0 Figures ................................................................................. 66
2.6.0 References .......................................................................... 74

## Chapter 3: Local dendrodendritic inhibition regulates fast synaptic transmission in visual thalamus
3.0.0 Abstract ............................................................................. 80
3.1.0 Introduction ......................................................................... 81
3.2.0 Methods and materials .......................................................... 83
3.3.0 Results ............................................................................... 87
3.4.0 Discussion .......................................................................... 96
3.5.0 Figures ................................................................................. 101
3.6.0 References .......................................................................... 109

## Chapter 4: Thalamic microcircuits: presynaptic dendrites form two distinct feedforward inhibitory pathways in thalamus
4.0.0 Abstract ............................................................................. 112
4.1.0 Introduction ......................................................................... 113
4.2.0 Methods and materials .......................................................... 115
4.3.0 Results ............................................................................... 119
4.4.0 Discussion .......................................................................... 128
4.5.0 Figures ................................................................................. 133
4.6.0 References .......................................................................... 139

## Chapter 5: Conclusion
5.0.0 Concluding remarks ............................................................... 143
5.1.0 References .......................................................................... 148
Chapter 1

Introduction

1.0.0. How inhibition shapes neural network activity

The brain consists of billions of individual neurons, all of which have similar but distinct physiological properties (Llinas, 1988). These neurons, in turn, assemble into neural networks, which are groups of individual neurons that communicate via chemical or electrical synaptic connection to perform specific physiological functions (Kandel et al., 2000; Purves et al., 2001). Ultimately, it is the coordinated neural activities generated within and between these different networks that underlie how the brain guides behavior, learns, and remembers. To begin understanding how neural networks operate, it is important to first understand the functional role of each neuron within the network and how they interact with each other (i.e., receive, encode, and communicate information).

Every network in the brain is made up of excitatory and inhibitory neurons (Kandel et al., 2000; Purves et al., 2001). Excitatory neurons or principal neurons comprise most of the neural population, while inhibitory neurons are the minority (Isaacson and Scanziani, 2011; Markram et al., 2004; McBain and Fisahn, 2001; Spruston, 2008). As shown in figure 1, the connection patterns between inhibitory neurons and excitatory neurons can be described in two ways (Isaacson and Scanziani, 2011). The first is feedback inhibition, in which the output of the principal cell can excite an inhibitory cell that then projects back upon the principal cell to provide inhibitory input. The second is feedforward inhibition, in which diverging excitatory afferents excite both an excitatory and inhibitory neuron which also innervates the same
excitatory neurons that received the divergent input. Together, these two simple inhibitory circuits form the basic circuitry of nearly all neural networks in the brain (Isaacson and Scanziani, 2011; Kandel et al., 2000). I believe understanding how these connection patterns relate to the functional organization of a network will ultimately provide valuable insight as to the role of inhibition in normal network operations. Moreover, I think understanding the various factors (i.e., intrinsic and extrinsic) that influence how inhibitory neurons receive, encode, and communicate information, will also shed light as to how inhibition is recruited during normal network operations.

1.1.0. Sensory processing and the thalamocortical circuit

The world is a beautiful and often dangerous place, full of numerous sights, sounds, tastes, and smells. To make sense of the world around us the brain must routinely organize and perceive different kinds of sensations. The brain uses sensory information not only to create a coherent representation of the world, but also to guide adaptive behaviors, improve learning and remembering, and develop complex motor and social skills (Carew, 2000; Kandel et al., 2000; Purves et al., 2001). Despite some understanding of the pathways and the first few brain regions involved in sensory processing, exactly how the brain is able to integrate such a wealth of information is poorly understood.

Sensation begins in the periphery, where specialized receptors are located and responsible for transducing and encoding information about a specific sensory stimulus (e.g., light or sound) (Kandel et al., 2000; Purves et al., 2001). This information is then transferred from the periphery to the neocortex, where sensory processing culminates. En route to the neocortex, all sensory information (except olfaction) must pass along the ascending pathway of the thalamocortical
circuit (Figure 2). A key feature of this circuit is the intimate, reciprocal synaptic connections made between associated thalamic and neocortical brain areas. Thalamocortical circuits constitute the vast majority of the mammalian brain and their activities play a critical role in a variety of brain functions including: sensory processing, behavioral state transitions, arousal, attention, plasticity, corticocortical communication, and certain pathophysiological conditions (Castro-Alamancos and Connors, 1996; Crunelli and Leresche, 2002; McCormick and Bal, 1997; Pinault, 2004; Sherman, 2007; Steriade et al., 1993b).

By virtue of its location and apparent simple anatomical layout, the thalamus has been traditionally viewed as a simple relay station for peripheral information destined for the neocortex. This is evident by the name relay neuron, given to the principal output cells of the thalamus. However, over the last 25 years, research has made it increasingly clear that the thalamus and its neurons are deeply involved in sensory processing (Sherman and Guillery, 1996). Thus, understanding how thalamic neurons collect and process sensory information prior to communicating to the neocortex will provide valuable insight regarding sub-cortical sensory processing in the brain.

1.2.0. The thalamus

The thalamus or diencephalon is a symmetrical structure, located at the midline and near the center of the brain (Kandel et al., 2000). It can be divided into four distinct groups: the epithalamus, the dorsal thalamus, the ventral thalamus, and the hypothalamus. Here, we are only concerned with the dorsal thalamus, which is the largest component of the diencephalon and most involved in sensory processing. The dorsal thalamus is the major extrinsic input to the
neocortex and provides virtually all its sensory input (Jones, 1985). In the following, the term “thalamus” means “dorsal thalamus”, unless indicated otherwise.

Anatomically, the thalamus can be subdivided into a number of distinct nuclei based on their inputs and outputs. There are three basic types of thalamic nuclei: first-order or relay nuclei, higher-order or association nuclei, and non-specific nuclei (Guillery and Sherman, 2002; Jones, 1985; Sherman, 2007). First-order or relay nuclei receive excitatory input from ascending sensory afferents and project their axons to distinct neocortical regions, specifically layer IV of primary sensory regions. Some of these nuclei include the dorsal lateral geniculate nucleus (dLGN: vision), the medial geniculate body (MGB: auditory) and the ventral posterior lateral/medial nuclei or ventral basal complex, (VPL/VPM or VB: somatosensory). Higher-order or association nuclei do not receive direct peripheral input but instead receive the bulk of their excitatory input from layer V of the neocortex. Like first-order nuclei, they also project their axons to distinct regions of the neocortex. Some of these nuclei include the mediodorsal nucleus (MD: prefrontal and limbic), the posterior nucleus (PO: somatosensory), and the pulvinar (visual cortex). Nonspecific nuclei receive ascending input from the reticular formation and project their axons broadly throughout the neocortex. These nuclei include the intralaminar and midline nuclei of the thalamus.

1.3.0. Thalamic relay neurons

The principal output neuron of the thalamus is the thalamocortical projection neuron or thalamic relay neuron (Sherman and Guillery, 1996). In first-order nuclei, thalamic relay neurons receive direct excitatory (i.e., glutamatergic) input from primary sensory afferents, which activate both AMPA and NMDA glutamate receptors (Hegglund and Hartveit, 1990; Liu, 1997;
Williams et al., 1996). Axon terminals of primary sensory afferents are known as RLP type terminals, because ultrastructurally they have round vesicles, large profile, and pale mitochondria (Erisir et al., 1998; Guillery, 1969). Anatomically, RLPs preferentially target proximal dendrites of thalamic relay neurons (Hamos et al., 1987; Wilson et al., 1984), suggesting sensory afferents strongly influence spike output of thalamic relay neurons.

Thalamic relay neurons, in turn, project their axons exclusively to the neocortical region responsible for processing the associated sensory information, also known as primary sensory cortices (Sherman and Guillery, 1996). For example, the visual thalamus or dLGN projects to the primary visual cortex or V1. In the neocortex excitatory axon terminals of thalamic relay neurons (i.e., the thalamocortical synapse) innervate layer IV neurons, the recipient layer of specific thalamic inputs in the neocortex (Castro-Alamancos and Connors, 1997). Within layer IV, relay neurons make direct excitatory connections with regular-spiking excitatory neurons, as well as fast-spiking interneurons, which form a disynaptic feedforward inhibitory connection with the regular-spiking neurons (Bagnall et al., 2011; Cruikshank et al., 2007; Inoue and Imoto, 2006).

Thalamic relay neurons also receive glutamatergic input from the neocortex (Sherman and Guillery, 1996). Neocortical input to first-order thalamic relay neurons originates from layer VI neurons of the associated neocortical brain region (e.g., V1 to dLGN). As mentioned above, these inputs are excitatory in nature (i.e., glutamatergic), so they activate AMPA and NMDA receptors, as well as metabotropic glutamate receptors (mGluRs) (Godwin et al., 1996; Liu, 1997; McCormick and von Krosigk, 1992). Neocortical axon terminals are classified as RSD terminals, because ultrastructurally they have round vesicles, small profile, and dark mitochondria (Erisir et al., 1997b, 1998; Guillery, 1969). Anatomically, RSDs preferentially target distal dendrites of thalamic relay neurons (Hamos et al., 1987; Wilson et al., 1984).
Thalamic relay neurons also receive inhibitory input from other thalamic neurons: thalamic interneurons and neurons of the thalamic reticular nucleus (TRN) (Pinault, 2004; Sherman and Guillery, 1996; Wang et al., 2011). Local interneurons are found throughout the thalamus and their axons project locally within the nucleus in which their soma is found (Sherman, 2004). In contrast, the TRN is a thin, shell-shaped nucleus that surrounds the dorsal and lateral parts of the thalamus (Pinault, 2004). TRN neurons, in turn, project their axons only into the thalamus, where they innervate virtually all the nuclei of the dorsal thalamus (Guillery and Harting, 2003). Both neurons are inhibitory in nature (i.e., GABAergic), so can activate both GABA_A and GABA_B receptors (Blitz and Regehr, 2005; Cox et al., 1997; Crunelli et al., 1988; Kim et al., 1997). Anatomically, inhibitory terminals are found targeting the entire dendritic arbor of thalamic relay neurons (Montero, 1986; Ohara et al., 1983; Wilson et al., 1984).

Lastly, thalamic relay neurons also receive a variety of neuromodulatory inputs, which originate from numerous brain stem areas (Sherman and Guillery, 1996). They include: cholinergic input from the parabrachial region, noradrenergic input from the locus coeruleus, serotonergic input from the dorsal raphe nucleus, and histaminergic input from the tubermammillary nucleus of the hypothalamus. These inputs can be either excitatory or inhibitory in nature, depending on their postsynaptic receptors, but perhaps more importantly these modulatory inputs produce relatively long-lasting effects that can alter the “state” of the thalamic neurons compared to other shorter effects of glutamate and GABA.

Surprisingly, considering that thalamic relay neurons are responsible for communicating sensory information to the neocortex, primary sensory afferents account for less than 10% of all the synaptic contacts on a given relay cell (Liu et al., 1995; Van Horn et al., 2000). The vast majority of synaptic inputs onto these neurons are non-primary sensory in origin (>90%). These
inputs consist of descending excitatory afferents from neocortex (~30%), ascending 
neuromodulatory inputs from the brainstem (~30%), and local innervation from inhibitory 
thalamic neurons (~30%). Given the sensory relay function of thalamic relay neuron, why have 
so few synaptic inputs from primary sensory sources? It is thought that these non-sensory inputs 
play an important role in modulating thalamic relay neuron excitability and thus the effectiveness 
of the sensory input to be transmitted to downstream targets (Sherman and Guillery, 2002). 
Understanding how non-sensory inputs modulate thalamic relay neuron excitability will provide 
valuable insight as to the functional role of the thalamus during sensory processing.

Physiologically, thalamic relay neurons display unique ionic conductances in their soma 
and dendrites, which allow them to discharge sodium-dependent action potentials in one of two 
modes: tonic or burst (Sherman, 2001). Transition from bursting to tonic firing is dependent on 
voltage-sensitive ionic conductances that are modulated with membrane potential (Jahnsen and 
Llinas, 1984a, b; Llinas and Jahnsen, 1982). At membrane potentials more positive than -60 mV, 
thalamic neurons are in tonic mode and respond to prolonged depolarization (i.e., excitation) 
with a repetitive discharge of sodium-dependent action potentials. In contrast, at membrane 
potentials more negative than -70 mV, thalamic neurons are in burst mode and respond to 
excitation with a slow depolarization that leads to a high-frequency burst discharge of sodium-
dependent action potentials. This slow depolarization or low-threshold spike (LTS) is an intrinsic 
membrane property of the relay neuron and is mediated by calcium (Jahnsen and Llinas, 1984b). 
The source of calcium underlying the LTS is from the activation of transient or T-type voltage-
gated calcium channels (Coulter et al., 1989; Crunelli et al., 1989; Huguenard and Prince, 1992). 
Thalamic relay neurons express high levels of T-type calcium channels, specifically the CaV3.1 
or α1G subunit (Talley et al., 1999). These T-type channels are unique from most other voltage-
gated calcium channels in that they are low-threshold activated, meaning they are inactive at relative depolarized membrane potentials but are de-inactivated at hyperpolarized membrane potentials, and are quickly inactivated giving rise to their transient current response (Huguenard, 1996). When in the de-inactivated state, sufficient depolarization of the membrane potential will cause T-type calcium channels to activate and subsequently inactivate, giving rise to their transient nature. Upon activation they produce a large calcium influx which leads to the generation of the LTS and burst discharge. In general, tonic mode (i.e., depolarized membrane state) has been associated with awake or alert behavioral states, while burst mode (i.e., hyperpolarized membrane state) is associated with sleep or non-alert behavioral states (Llinas and Steriade, 2006; McCormick and Bal, 1997; Steriade et al., 1993b). Nonetheless, burst firing has been observed in awake and behaving animals (Guido and Weyand, 1995; Ramcharan et al., 2000), therefore could serve as an effective mode for relaying sensory information to the neocortex.

1.4.0. Local thalamic interneurons

As mentioned above, thalamic interneurons are a major source of inhibition in the thalamus (Sherman, 2004; Sherman and Guillery, 1996). Thalamic interneurons are found throughout the dorsal thalamus and their axons project locally within the nucleus that their soma is found. In the cat dLGN, interneurons have been described as small cells having compact dendritic and axonal arbors that are oriented perpendicular to the laminar borders (Famiglietti and Peters, 1972; Guillery, 1966; Hamos et al., 1985). Although it is not entirely clear if there is a heterogeneous population of thalamic interneurons, there is evidence in the cat dLGN
suggesting at least two populations based on nitric oxide synthase content, synaptic connections, and morphology (Bickford et al., 1999; Famiglietti, 1970; Updyke, 1979).

Surprisingly, the presence or total number of interneurons found within a given thalamic nucleus can differ considerably between mammalian species (Arcelli et al., 1997). For example, in the rat thalamus, the dLGN has interneurons but the VB nucleus does not. In contrast, in the cat thalamus, interneurons are not only found in both the dLGN and the VB but they also account for a larger proportion of the total neuronal population within a given nucleus than that of the rat. The reason for such diversity across species is unclear. However, it has been suggested that the presence of interneurons do not reflect the ability to perform specific tasks, but likely reflect the complexity of sensory processing occurring at the level of the thalamus (Arcelli et al., 1997). Overall, in nuclei in which they are found, GABAergic interneurons typically comprise ~15-30% of the total neuronal population (Arcelli et al., 1997).

Unlike many interneurons of the central nervous system, thalamic interneurons are unique in that they can inhibit neighboring neurons with not only traditional axonal output but with specialized dendrites that serve both pre and postsynaptic roles (Famiglietti and Peters, 1972; Guillery, 1969; Hamos et al., 1985; Montero, 1986; Ralston, 1971; Sherman, 2004). With traditional axonal output, sodium dependent action potentials travel along the axon, resulting in GABA release from axonal terminals. Based on their ultrastructure, these axonal terminal of inhibitory neurons are termed F1 terminals, since their vesicles are densely packed and appear flat or pleomorphic in shape (Wilson et al., 1984). In contrast, dendritic terminals of thalamic interneurons are termed F2 terminals, since their vesicles are loosely packed, appear flat or pleomorphic in shape, are localized within dendrites, and are found presynaptic to relay neuron dendrites (Famiglietti and Peters, 1972; Wilson et al., 1984). Since dendrites are the primary
target of excitatory afferents, neurons with presynaptic dendrites are thought to produce output local to the site of excitation and independent of the soma-axon (Margrie and Urban, 2008). This is thought to be the case for thalamic interneurons (Bloomfield and Sherman, 1989).

Like thalamic relay neurons, thalamic interneurons also receive excitatory input from primary sensory afferents (Sherman, 2004). For example, in the visual thalamus, retinogeniculate afferents target both excitatory relay neurons that project to layer IV visual cortex and inhibitory interneurons that project to relay neurons and each other (Hamos et al., 1985; Lieberman, 1973; Montero, 1986, 1991). The GABAergic connections made by thalamic interneurons, in turn, form a retinogeniculate feedforward inhibitory circuit onto the relay cell. In addition to excitatory input from sensory afferents, thalamic interneurons also receive glutamatergic input from the neocortex and the axon collaterals of thalamic relay neurons (Acuna-Goycolea et al., 2008; Augustinaite et al., 2011; Bickford et al., 2008; Errington et al., 2011; Govindaiah and Cox, 2004; Hamos et al., 1985; Williams et al., 1996). Since all these terminals are glutamatergic in nature, they activate AMPA and NMDA glutamate receptors (Liu, 1997). mGluRs are also found postsynaptic to sensory afferents on thalamic interneurons (Godwin et al., 1996; Govindaiah and Cox, 2004). Thalamic interneurons also receive cholinergic input from the parabrachial region of the brainstem (Erisir et al., 1997b). These afferents activate Type 2 muscarinic receptors (M2) but not the ionotropic nicotinic receptor (nACh), resulting in a hyperpolarization in interneurons (Carden and Bickford, 1999; Cox and Sherman, 2000; Pape and McCormick, 1995; Plummer et al., 1999).
1.4.1. The triadic arrangement

As mentioned above, thalamic interneurons have specialized dendrites that serve both presynaptic and postsynaptic roles (Famiglietti and Peters, 1972; Guillery, 1969; Hamos et al., 1985; Montero, 1986; Ralston, 1971; Sherman, 2004). Moreover, since dendritic terminals are positive for glutamic acid decarboxylase (GAD) (Montero, 1986), their output is GABAergic and thus inhibitory in nature. Because the vast majority of work examining thalamic interneurons has focused on the visual thalamus, we will from this point refer to them with respect to the circuitry of the dLGN. Nonetheless, limited work suggests that the same properties hold true for interneurons in other thalamic nuclei (Sherman, 2004). As shown in figure 3, F2 terminals are often found in triads, a unique synaptic arrangement in which an interneuron dendrite is presynaptic to a relay neuron dendrite and postsynaptic to an excitatory retinogeniculate terminal that, in turn, targets the same postsynaptic relay dendrite (Hamos et al., 1985; Wilson et al., 1984). Most triads are also located within a glomerulus, a glia process that is not juxtaposed to individual synapses but instead encloses the entire circuitry (Famiglietti and Peters, 1972). The exact functional significance of the glomerulus is unknown, but it might serve to regulate the extent of neurotransmitter spread. However, it is important to note that not all F2 terminals in synaptic triads (Dankowski and Bickford, 2003; Datskovskaia et al., 2001).

Most F2 terminals are located on distal dendrites of interneurons (Ralston, 1971), where they can be found on small dendritic appendages (with thin stalks) and not on dendritic trunks (Guillery, 1969; Hamos et al., 1985; Montero, 1986, 1991; Ralston, 1971). Moreover, anatomical studies indicate that not only are dendritic appendages of interneurons the primary target of retinogeniculate terminals but, most F2 terminals are postsynaptic to RLP terminals originating from the retina (Guillery, 1969; Hamos et al., 1985; Montero, 1986; Ohara et al., 1983). F2
terminals are also commonly found postsynaptic to cholinergic input originating from the parabrachial region of the brainstem (Erisir et al., 1997a).

Although F2 terminals can innervate other interneurons, they are predominantly found presynaptic to thalamic relay neurons (Hamos et al., 1985). On relay neurons, retinogeniculate terminals, and thus F2 terminals, are preferentially localized to the proximal dendrites of relay neurons (Hamos et al., 1987; Wilson et al., 1984). Moreover, as described in the cat dLGN, synaptic triads are often located on ‘grape-like’ appendages near branch points on relay neurons (Friedlander et al., 1981; Wilson et al., 1984). The purpose for targeting dendritic appendages on relay neurons is unknown.

### 1.4.2. Regulation of F2 output

Since most F2 terminals are postsynaptic to excitatory afferents (Hamos et al., 1985), glutamate must play a major regulatory role in dendrodendritic communication in the thalamus. Anatomical work shows that the presynaptic dendrites of thalamic interneurons are immunoreactive for not only GluR2/3 and NMDAR1 receptor subunits but also the Type 5 metabotropic glutamate receptor (mGluR5) (Godwin et al., 1996; Liu, 1997). Interestingly, the mGluR5 label is prominent in inhibitory F2 terminals associated with retinal input to thalamic relay neurons (Godwin et al., 1996). Pharmacological activation of mGluR5, via bath application of receptor agonists, results in a robust increase in inhibitory activity recorded in postsynaptic relay neurons (Cox and Sherman, 2000; Cox et al., 1998; Errington et al., 2011; Govindaiah and Cox, 2006; Lam et al., 2005). Since mGluR5 agonists produce no action potential output from interneurons and the change in inhibitory activity is insensitive to voltage-gated sodium channels blockers (i.e., tetrodotoxin: TTX), the activity must therefore be the result of local activation of
dendritic F2 terminals on thalamic interneurons. It has also been demonstrated that high frequency stimulation of the optic tract, but not the optic radiation, can result in the mGluR mediated increase in F2 output (Govindaiah and Cox, 2004), suggesting retinal afferents are the driving input to F2 terminals. There is also data to suggest AMPA and NMDA receptors can also regulate F2 terminal output (Acuna-Goycolea et al., 2008; Blitz and Regehr, 2005). However, since these studies have relied on widespread synaptic stimulation in order to activate F2 terminals, action potential mediated F1 terminal activity could not be ruled out.

Since iGluRs and mGluRs have vastly different time course and activation characteristics (Anwyl, 1999; Madden, 2002), F2 terminal output could be activity-dependent if regulated by both classes of glutamate receptors. Thus, iGluR and mGluR mediated F2 output could result in both short-term and long-term feed-forward inhibition, respectfully. Although the function of the F2 terminal is unknown, it has been hypothesized that mGluR-mediated output would provide an excellent gain control for relay neurons, keeping the transfer of information from thalamus to neocortex within the linear regime (Sherman, 2004). In contrast, the faster ionotropic-mediated output would likely control the temporal window for sensory inputs to integrate with other excitatory inputs (Cruikshank et al., 2007; Pouille and Scanziani, 2001; Spruston et al., 2008), ultimately affecting the probability of a single excitatory retinogeniculate input to contribute to action potential output.

Recent work has also shown that the dendrites of thalamic interneuron have active conductances which could also facilitate GABA release from F2 terminals (Acuna-Goycolea et al., 2008; Casale and McCormick, 2011). The presence of voltage-gated sodium channels allow action potentials initiated at the soma (not in the dendrites) to propagate not only down the axon but back into the dendrites (Casale and McCormick, 2011). These backpropagating action
potentials invade small distal dendrites where they presumably release GABA from dendritic F2 terminals (Acuna-Goycolea et al., 2008; Casale and McCormick, 2011). Moreover, the dendrites of thalamic interneuron appear to also generate L-type mediated plateau-like calcium spikes (Acuna-Goycolea et al., 2008). Dendritic calcium spikes are generated in the dendrites and occur only when the interneuron membrane is hyperpolarized and the neuron receives synchronous input from multiple retinogeniculate axons. Once initiated, these long-lasting calcium spikes propagate throughout the dendritic arbor where they presumably result in global release of GABA from the presynaptic dendrites of interneurons. Interestingly, plateau-like calcium spikes have also been observed after high frequency stimulation of corticothalamic fibers (Augustinaite et al., 2011), suggesting the cortex could also drive dendritic output.

As mentioned above, F2 terminals also receive cholinergic input from the parabrachial region of the brainstem (Erisir et al., 1997a). Unlike excitatory afferents, muscarinic agonists can lead to a global reduction in inhibitory activity in thalamic relay neurons (Cox and Sherman, 2000). The sensitivity of inhibitory activity to muscarinic receptor activation is consistent with anatomical work showing F2 terminals are immunoreactive for the M2 receptor (Carden and Bickford, 1999; Plummer et al., 1999). Since increased cholinergic output from the parabrachial regions is related to heightened arousal states (Steckler et al., 1994), the release of acetylcholine would likely suppress inhibition in relay neurons during certain behavioral states. However, recent research suggests that cholinergic input could increase F2 terminal output in a manner that is dependent on the level of afferent activity (Antal et al., 2010). Since activation of M2 receptors leads to the hyperpolarization of presynaptic dendrites, this change in membrane potential will facilitate the activation of plateau-like dendritic calcium spikes in interneuron dendrites (Acuna-Goycolea et al., 2008; Antal et al., 2010). This calcium spike will, in turn,
result in global dendritic release. These data imply that cholinergic input will reduce feedforward F2 terminal inhibition in relay neurons in response to a few retinogeniculate inputs, thereby making relay neurons more responsive to weak inputs. In contrast, when a large number of retinogeniculate inputs are activated, cholinergic input will increase F2 terminal mediated inhibition in relay neurons by generating a global calcium spike in interneuron dendrites, thereby making relay neurons less responsive to strong inputs.

1.4.3. Thalamic interneuron physiology

Physiologically, thalamic interneurons are different from thalamic relay neurons (Pape et al., 1994; Pape and McCormick, 1995; Turner et al., 1997; Williams et al., 1996). Perhaps the most striking difference is how they respond to a depolarization when held at a hyperpolarized membrane potential (i.e., burst mode). Unlike relay neurons, thalamic interneurons do not generate a well-defined burst of action potentials (Pape and McCormick, 1995; Williams et al., 1996), which can range from a small subthreshold response to the generation of one or few action potentials. Although interneurons appear to have T-type calcium current, the current appears masked by A-type potassium current that activates around the same potential (Pape et al., 1994; Pape and McCormick, 1995). Blocking A-type potassium current can result in a larger calcium current but the overall magnitude is still smaller than that seen in relay neurons (Pape et al., 1994; Pape and McCormick, 1995). However, there has been studies in which robust bursts in thalamic interneurons have been observed (Zhu and Heggelund, 2001; Zhu et al., 1999a; Zhu et al., 1999b). Technical and procedural differences were said to account for the difference from those of previous studies. If interneurons do generate burst output, it would imply their output is
state-dependent and would suggest they could play a role in network oscillations (Zhu et al., 1999a; Zhu et al., 1999b).

1.4.4. Function of local thalamic interneurons

Functionally, inhibition in the dLGN is thought to enhance stimulus selectivity, improve sensory coding, and ensure temporal precision of spiking (Wang et al., 2011). However, the function of inhibitory F2 terminals in visual processing remains unresolved. Currently, the leading hypothesis suggest that mGluR-mediated F2 output modulates the balance between excitation and inhibition within the triad, therefore reducing the gain of coupled retinogeniculate terminals (Sherman, 2004). The shift in gain would likely reduce the strength of the synapse and decrease the probability of coupled retinogeniculate terminals to contribute to relay neuron spiking.

Alternatively, it has been proposed that thalamic interneurons may behave as multiplexing integrators (Bloomfield and Sherman, 1989; Cox et al., 1998). When interneurons were first identified as having presynaptic dendrites, it was thought that distal synaptic activity would be insufficient for action potential generation at the soma but could be sufficient for generating local transmitter release from the dendrite (Ralston, 1971). This was based on the close proximity of excitatory afferents to the F2 terminals and the passive, cable-like membrane properties of dendrites, which would significantly attenuate the summation and propagation of any signal generated distally (Rall, 2008; Spruston et al., 2008). In fact, computational work supports the notion that the dendrites of thalamic interneurons are electrically isolated from one another and the soma/axon (Bloomfield and Sherman, 1989). Moreover, physiological work also suggests that dendritic output is independent of the soma/axon (Acuna-Goycolea et al.,
However, these studies have relied on widespread pharmacological and synaptic stimulation in order to activate F2 terminals. Thus, both approaches result in global activation of the entire dendritic arbor and make it impossible to assay whether F2 terminals produce more localized input-output computations. If F2 terminals can behave locally and independently, this suggests that thalamic interneurons can behave as multiplexing integrators, with numerous independently operating input-output devices. Such a feature is qualitatively similar to that described for amacrine cells in the retina, where neurotransmitter release from dendrites is believed to be isolated and reflects the local computation of visual information (Euler et al., 2002; Grimes et al., 2010).

1.5.0. The thalamic reticular nucleus

In addition to thalamic interneurons, neurons of the thalamic reticular nucleus (TRN) also provide a major source of inhibition in the thalamus. The TRN is a thin shell-shaped nucleus located at the interface of the thalamus and neocortex, just medial to the white matter tract that creates the internal capsule (Jones, 1985; Kolliker, 1896; Pinault, 2004). It develops from the ventral thalamus to ultimately surround the dorsal and lateral parts of the thalamus (Jones, 1985; Rose, 1942). By virtue of its location, all axons of the thalamocortical pathway transverse the TRN, linking it with numerous brain regions (Jones, 1975; Rose and Woolsey, 1949).

As a consequence of its location, the TRN was originally believed to be an extension of the brainstem reticular formation, this was because it appeared TRN neurons had axons with widespread neocortical projections and thus global actions (Jasper, 1949). However, it is now known that the TRN does not project to the neocortex (Carman et al., 1964; Chow, 1952; Rose,
1952), but receive widespread neocortical and thalamic input (Jones, 1975). In turn, TRN neurons project their axons into the dorsal thalamus (Cajal, 1995; Scheibel and Scheibel, 1966).

As shown in figure 4a, associated thalamocortical and corticothalamic axon fibers converge at the TRN in the same location, creating dense zones of terminal innervation (Guillery and Harting, 2003). These zones or sectors create a crude topographical organization within the TRN based on the information carried by the associated fibers (e.g., visual sector - visual information). Furthermore, each TRN sector can be further divided into 2-3 slabs or tiers that lie parallel to the plan of the nucleus (Crabtree, 1996, 1998, 1999) (Figure 4b). Slabs receive more focal innervation from their associated areas (i.e., thalamus and neocortex) and project their axons to discrete regions of associated thalamic nuclei (i.e., First-order or Higher-Order) (Crabtree, 1999; Pinault, 2004). In total, seven sectors have been identified within the TRN, each relating to a different functional modality: somatosensory, auditory, visual, gustatory, motor, limbic/prefrontal, and visceral (Cicirata et al., 1990; Conley and Diamond, 1990; Crabtree, 1996, 1998; Hayama et al., 1994; Lozsadi, 1994; Stehberg et al., 2001). This unique structural organization could allow the TRN to behave as an “integrating hub”, capable of sampling and modulating the flow of information through numerous thalamocortical circuits.

1.5.1. Inputs to the thalamic reticular nucleus

As mentioned above, the TRN receives afferent input from both thalamus and neocortex. As thalamocortical and corticothalamic axons transverse the TRN, they give rise to collaterals that make synaptic contacts onto dendrites of TRN neurons (Bourassa et al., 1995; Jones, 1985; Liu, 1997). These afferent inputs are excitatory in nature, activating both AMPA and NMDA receptors as well as mGluRs (Cox and Sherman, 1999; Cruikshank et al., 2010; Golshani et al.,
Corticothalamic fibers originate from deep layer VI neocortical neurons and form small, RSD terminals on TRN dendrites (Bourassa et al., 1995; Guillery, 1969; Williamson et al., 1993). In contrast, thalamocortical axons form large synaptic terminals (Ohara and Lieberman, 1985). Quantitative anatomical studies suggest that the majority (>60%) of the synaptic contacts onto TRN neurons are from the neocortex (Liu and Jones, 1999). Moreover, their distribution suggest corticothalamic terminals favor distal dendrites, whereas thalamocortical terminals favor proximal dendrites (Liu and Jones, 1999).

In addition to thalamic and neocortical input, the TRN also receives numerous inputs from various brain regions (McCormick and Bal, 1997). Overall, these inputs serve to modulate the overall excitability of TRN neurons and display a less discrete organization than thalamocortical and corticothalamic inputs. The TRN receives inhibitory GABAergic inputs from the globus pallidus (Gandia et al., 1993), the substantia nigra pars reticulate (Pare et al., 1990), the caudal basal forebrain (Asanuma and Porter, 1990), and the zona incerta (Cavdar et al., 2006). Cholinergic inputs from the basal forebrain and brainstem are also inhibitory (Hallanger et al., 1987; McCormick and Prince, 1986). However, acetylcholine has been shown to excite TRN neurons via postsynaptic nicotinic receptors (Lee and McCormick, 1995). Histaminergic input from the hypothalamus also inhibits TRN neurons (Manning et al., 1996). In contrast, noradrenergic input from the locus coeruleus and serotonergic input from the dorsal raphe nucleus excite TRN neurons (Asanuma, 1992; Cropper et al., 1984; McCormick and Wang, 1991). Dopamine increases TRN excitability by inhibiting GABAergic terminals originating from the globus pallidus (Govindaiah et al., 2010). Lastly, there are neuropeptides (e.g., cholecystokinin and vasoactive intestinal peptide) that modulate TRN excitability (Cox et al., 1995; Lee and Cox, 2003, 2006). Together, these various neuromodulators appear to
modulate the overall excitability of TRN neurons (McCormick and Bal, 1997; Steriade et al., 1993b).

There is also evidence that TRN neurons communicate with each other. Intra-TRN communication can be chemical in nature (i.e. inhibitory), via axodendritic and possibly dendrodendritic synapses (Deschenes et al., 1985; Huntsman et al., 1999; Pinault et al., 1997; Sanchez-Vives et al., 1997; Scheibel and Scheibel, 1966; Sohal and Huguenard, 2003; Sun et al., 2011; Zhang and Jones, 2004). In addition, TRN neurons can communicate via electrical synapses (Haas and Landisman, 2012; Haas et al., 2011; Landisman et al., 2002; Long et al., 2004), formed by the gap junction proteins connexin-36 (Cx36) (Liu and Jones, 2003). Together, intra-TRN communication appears to play an important role in regulating oscillations in the thalamocortical network (Long et al., 2004; Steriade et al., 1985).

1.5.2. TRN innervation of the dorsal thalamus

The TRN innervates virtually all the nuclei of the dorsal thalamus (Cox et al., 1996; Gonzalo-Ruiz and Lieberman, 1995; Kolmac and Mitrofanis, 1997; Pinault et al., 1995; Pinault and Deschenes, 1998; Yen et al., 1985). Since all TRN neurons are GABAergic in nature (Houser et al., 1980), their output alters thalamic relay neuron excitability (Cox et al., 1997; Kim et al., 1997). In addition, inhibition arising from TRN neurons has been shown to alter the receptive field properties of thalamic relay neurons (Lee et al., 1994a, b).

All TRN neurons have a single axon that emerges from the soma/proximal dendrite and enters the dorsal thalamus in a ventromedial direction. In general, TRN axons innervate only the ipsilateral side of the dorsal thalamus, however there is a report suggesting some may target the contralateral side (Chen et al., 1992). Occasionally, an axon can split into two independent
branches, targeting either a single nucleus or two distinct nuclei (Pinault et al., 1995; Pinault and Deschenes, 1998). Sometimes axons produce short-range collaterals, which remain within the TRN, presumably where they target other TRN neurons (Cox et al., 1996; Pinault et al., 1997; Scheibel and Scheibel, 1966). TRN neurons also display heterogeneous axonal arborizations, with three distinct patterns: cluster/compact, intermediate, and diffuse (Cox et al., 1996). Differences in axon arborization may reflect a dynamic range of inhibitory influence TRN has on thalamic activity.

1.5.3. Morphology of TRN neurons

TRN neurons have elongated dendrites that stretch parallel to plane of the nucleus (Lubke, 1993; Ohara and Havton, 1996; Scheibel and Scheibel, 1966; Spreafico et al., 1991). Each neuron has 3-6 primary dendrites, typically originating from the poles of a fusiform-shaped cell body. Dendrites have a relatively smooth appearance and can have both axon-like varicosities and thin hair-like processes (Lubke, 1993; Pinault et al., 1997; Scheibel and Scheibel, 1966). The varicosities appear as ‘puncta adherentia’, which are small, specialized junctions, thought to be dendrodendritic terminals (Ohara and Lieberman, 1985; Pinault et al., 1997). Dendrites can be as long as 200-400 \( \mu \)m, indicating that the dendritic field of some TRN neurons can span 400-800 \( \mu \)m in diameter.

Based on morphology, it is unclear if the TRN consists of a heterogeneous population. In one study, three morphological sub-types were identified (Spreafico et al., 1991). These included (1) a small fusiform type; (2) a large fusiform type; and (3) a small round type that was multipolar. However, numerous other studies have reported no clear differences (Lubke, 1993; Ohara
and Havton, 1996; Scheibel and Scheibel, 1966). If there is heterogeneity, it remains to be seen if such heterogeneity applies to any physiological characteristics and thus different functions.

### 1.5.4. Electrophysiological properties of TRN neurons

Like many other thalamic neurons, TRN neurons are endowed with unique intrinsic physiological properties which allowed them fire action potentials in one of two modes: tonic or burst (Avanzini et al., 1989; Jahnsen and Llinas, 1984a, b; Llinas and Jahnsen, 1982; Sprefico et al., 1988). When at a relatively depolarized membrane potential (greater than -65 mV), TRN neurons are in Tonic Mode and will respond to excitatory input with a repetitive discharge of sodium-dependent action potentials that will persisted through the excitation. Tonic mode is typically associated with the awake, alert behavioral state (Llinas and Steriade, 2006). In contrast, when at a relatively hyperpolarized membrane potential (less than -75 mV), TRN neurons are in Burst Mode and will respond to excitatory input with a transient, high-frequency burst of sodium-dependent action potentials. Burst mode is typically associated with the sleep, non-alert behavioral state (Llinas and Steriade, 2006). The transition between firing modes depends on the voltage-state of the transient-type (T-type) calcium channel (Crunelli et al., 1989; Hille, 2001; Huguenard, 1996; Huguenard and Prince, 1992). At depolarized potentials, T-type calcium channels are inactivated. However, when at hyperpolarized potentials these channels become de-inactivated and are capable of activating with sufficient depolarization. Once activated these channels quickly inactivate, giving rise to their transient nature. While activated they produce a transient inward calcium current ($I_T$), which generates a low-threshold spike (LTS). It is the LTS that underlies the burst response. Unlike relay neurons, which express the Ca$_{v}$.3.1($\alpha_{1C}$) T-Type calcium channel subunit, TRN neurons expresses high levels of the
Cav3.2($\alpha_{1H}$) and Cav3.3($\alpha_{1I}$) subunits (Talley et al., 1999). Interestingly, the Cav3.3 subunit has the slowest activation and inactivation kinetics of the three isoforms (Perez-Reyes, 2003). The unique properties of this isoform likely contribute to the longer-duration LTS and subsequently prolonged burst-discharge (i.e., more action potentials) observed in TRN when compared to relay neurons (Domich et al., 1986; Huguenard and Prince, 1992).

Recordings of TRN neurons \textit{in vivo} indicate they have large receptive fields when compared to thalamic relay neurons (Pollin and Rokya, 1982). This is consistent with the idea that TRN neurons are integrators, receiving converging information from multiple relay neurons. \textit{In vivo} recordings also suggest there is a sensory map across the TRN (Shosaku et al., 1984). Although the majority of TRN neurons clearly respond to a single modality, there have been some reports indicating that a small population of neurons can respond to more than one modality (Shosaku and Sumitomo, 1983; Sugitani, 1979). Their response latencies are generally slower than those observed in relay neurons, consistent with the fact they do not receive direct input from sensory afferents (Hartings et al., 2000; Sugitani, 1979). Under urethane-anesthesia, three response patterns have been observed in the TRN: On-Tonic, ON-Phasic, and ON-OFF-Phasic (Sugitani, 1979).

\textbf{1.5.5. TRN Function and role during attention}

Functionally, the TRN has been shown to alter thalamic relay neuron excitability as well as their receptive field properties (Cox et al., 1997; Kim and McCormick, 1998; Lee et al., 1994a, b). The TRN has also been implicated in the generation and maintenance of various synchronous rhythmic brain activities including: slow-wave sleep, spindle-wave sleep, and certain seizure states (Llinas and Steriade, 2006; McCormick and Bal, 1997; Steriade, 2005;
Steriade et al., 1993a; Steriade et al., 1986; Steriade et al., 1987; Steriade et al., 1993b). It may also serve as a hub for intra-thalamic communication between different thalamic nuclei (Crabtree, 1999; Crabtree et al., 1998; Crabtree and Isaac, 2002). In addition, given its structural organization and unique position at the interface of the thalamus and neocortex, the TRN has long been speculated to serve a role in selective attention (Crick, 1984; Yingling and Skinner, 1976).

In 1984, Francis Crick proposed a speculative hypothesis in which he believed the TRN could serve as an internal “searchlight”, focusing the brain’s attention on specific parts of sensory world for further analysis. He said, “If the thalamus is the gateway to the cortex, the TRN might be described as the guardian of the gateway.” His hypothesis suggested that the TRN would result in an increased thalamocortical communication through a post-anodal exaltation of thalamic relay neurons, which is an inhibition-induced hyperpolarization followed by a rebound burst discharge. Although, burst firing has a significant impact on neuron-neuron communication (Lisman, 1997), bursting in the thalamus is infrequent during the waking-state (McCormick and Bal, 1997; Steriade et al., 1993b). Given the infrequent nature of burst firing in the thalamus, some have speculated that bursts could serve as a “wake-up call” for the neocortex, facilitating signal transmission during target acquisition (Guido and Weyand, 1995). To date, whether the TRN serves as an internal “searchlight” that selectively enhances thalamocortical communication remains speculative.

However, there is evidence suggesting TRN activity is associated with attentional related tasks. Using the Fos-protein as a marker of TRN activity, it has been shown that distinct parts of the nucleus are activated during normal exploratory behavior of a novel and complex environment (Montero, 1997). The selective activation of different parts of the TRN appears
dependent on neocortical feedback and not sensory input, as focal lesions to the neocortex will reduce the level of Fos-activity during exploratory behavior (Montero, 2000). Moreover during classical-conditioning tests, selective Fos-activity can be observed in the TRN sector (e.g., visual) associated with the attended conditioned stimuli (e.g. light) when compared to the sector associated with the unconditioned stimuli (e.g. tone) (McAlonan et al., 2000). Together, these behavioral studies suggest that TRN activity is strongly correlated with the sensory modality being used during attentional-demanding tasks.

More recently, *in vivo* electrophysiological studies have shown that response properties of individual TRN neurons are selectively modulated by the degree of attention directed toward one of two sensory cues (i.e., auditory or visual) (McAlonan et al., 2006, 2008). Moreover, others have shown that auditory TRN neurons will rapidly adapt when continually presented with the same stimuli (Yu et al., 2009a). Interestingly, adaptation seems to be stimulus dependent, as subsequent presentation of a novel stimulus will result in a strong response recorded from the same neuron (Yu et al., 2009b). This suggests that TRN neurons could behave as salient detectors. Together, these studies provide convincing evidence that TRN activity is associated with the attentional state of the animal.

**1.6.0. Closing remarks**

Inhibition plays an essential role in modulating thalamic excitability and therefore communication between thalamus and neocortex. In thalamus, there are two principal sources of inhibition, thalamic reticular neurons and thalamic interneurons. Although the precise role of these neurons is still unclear, it has been postulated that their output acts to enhance stimulus selectivity, improve sensory coding, and ensure temporal precision of spiking of thalamic relay
neurons. To date, most research examining inhibition in the thalamus has focused on understanding the intrinsic physiology of inhibitory thalamic neurons and the influence of their output on postsynaptic relay neurons. However, when I began my graduate work I thought it might be worth taking a step-back and ask, not how inhibition shapes thalamic excitability but, how are inhibitory thalamic neurons deciding when it is appropriate to generate output. That is, how are inhibitory thalamic neurons integrating synaptic information? Specifically, I was interested in the intrinsic and extrinsic factors that allowed individual neurons to better encode and process incoming signals, so they can more accurately communicate information to downstream network targets. I believe understanding how these neurons integrate afferent information to produce output will ultimately provide valuable insight into the function of inhibition during normal network operations.

The work in this dissertation demonstrates that inhibitory thalamic neurons are not only uniquely positioned to modulate thalamic excitability, but the unique structural and intrinsic properties of their dendrites likely play a critical role in this function. Specifically, work in this dissertation focused on how the dendrites of thalamic reticular neurons and thalamic interneurons integrate excitatory afferent information. The data presented suggest 1) that dendritic T-type calcium channels boost distal afferent activity in thalamic reticular neurons and 2) that the presynaptic dendrites of thalamic interneurons behave as independent input-output devices. The experimental approaches used include a combination of in vitro electrophysiology, two-photon microscopy, calcium imaging, and glutamate uncaging.
Figure 1: Connection patterns between inhibitory neurons and excitatory neurons. **A**, Shown is a simple diagram illustrating the basic circuitry underlying feedback inhibition. Here, an inhibitory neuron receives excitatory input from a nearby principle neuron which and then projects back onto the same neuron. **B**, Shown is a simple diagram illustrating the basic circuitry underlying feedforward inhibition. Here, diverging excitatory afferents excite both an excitatory and inhibitory neuron which, in turn, projects back onto the same excitatory neurons that received the divergent input.
Figure 2: The thalamocortical circuit. A, Shown is a simple diagram illustrating the basic circuitry of the thalamocortical circuit. Here, excitatory thalamic and neocortical afferents link associated thalamic and neocortical brain regions. Inhibitory neuron in the thalamus (i.e., thalamic reticular neurons and thalamic interneurons) modulate thalamic excitability through both feedback and feedforward inhibitory circuits.
Figure 3: The synaptic triad. A. The basic circuitry of a triad located within a glomerulus. A retinogeniculate (RG) terminal forms an excitatory synapse onto both a thalamocortical (TC) relay neuron and interneuron dendrite (INT: F2 terminal). In turn, the interneuron dendrite (F2 terminal) forms an inhibitory synapse onto the same TC dendrite.
Figure 4: The functional organization of the thalamic reticular nucleus. **A**, Schema to show the thalamic reticular nucleus divided into sectors, sectors are innervated by associated neocortical and thalamic fibers. Reticular neurons, in turn, project their axons into the thalamus where they inhibit thalamic relay neurons excitability. **B**, Schematic illustrating how individual sectors can be further divided into three parallel slabs based on the position individual neurons. Neurons in each slab receive afferent input from distinct sub-regions within the thalamus or neocortex, as well as project their axons to distinct regions within the thalamus (not show).
1.8.0. References


Rose, J.E. (1942). The ontogenetic development of the rabbit's diencephalon. The Journal of Comparative Neurology 77, 61-129.


Chapter 2

Low-threshold Ca\(^{2+}\) current (I\(_T\)) amplifies distal dendritic signaling in thalamic reticular neurons\(^1\)

2.0.0. Abstract

The low-threshold transient calcium current (I\(_T\)) plays a critical role in modulating the firing behavior of thalamic neurons; however, the role of I\(_T\) in the integration of afferent information within the thalamus is virtually unknown. We have used two-photon laser scanning microscopy coupled with whole-cell recordings to examine calcium dynamics in the neurons of the strategically located thalamic reticular nucleus (TRN). We now report that a single somatic burst discharge evokes large magnitude calcium responses, via I\(_T\), in distal TRN dendrites. The magnitude of the burst-evoked calcium response was larger than those observed in thalamocortical projection neurons under the same conditions. We also demonstrate that direct stimulation of distal TRN dendrites, via focal glutamate application and synaptic activation, can locally activate distal I\(_T\) producing a large distal calcium response independent of the soma/proximal dendrites. These findings strongly suggest that distally located I\(_T\) may function to amplify afferent inputs. Boosting the magnitude ensures integration at the somatic level by compensating for attenuation that would normally occur due to passive cable properties. Considering the functional architecture of the TRN, elongated nature of their dendrites, and robust dendritic signaling, these distal dendrites could serve as sites of intense intra/cross-modal integration and/or top-down modulation leading to focused thalamocortical communication.

\(^1\) The work included in this chapter has been previously published in the Journal of Neuroscience and permission to reprint the material has been provided by the publisher. Crandall, S.R., Govindaiah, G., and Cox, C.L. (2010). Low-threshold Ca\(^{2+}\) current amplifies distal dendritic signaling in thalamic reticular neurons. J Neurosci 30, 15419-15429. Author contributions: S.R.C. and C.L.C. designed research; S.R.C. and G.G. performed research; S.R.C. analyzed data; S.R.C. and C.L.C. wrote the paper.
2.1.0. Introduction

The GABAergic thalamic reticular nucleus (TRN) plays a central role in modulating information transfer between thalamus and neocortex, as well as within the thalamus (Crabtree et al., 1998; Crabtree and Isaac, 2002; Crick, 1984; Steriade et al., 1986; Steriade et al., 1993; Yingling and Skinner, 1976). Centrally located in the thalamocortical circuit, corticothalamic and thalamocortical axons pass through the TRN providing the primary sources of synaptic inputs to the nucelus (Guillery and Harting, 2003; Jones, 1975; Liu and Jones, 1999; Ohara and Lieberman, 1981). In turn, TRN axons project into the thalamus where they modify thalamic activities such as neuronal excitability (Cox et al., 1997; Kim et al., 1997) and receptive field properties (Lee et al., 1994a, b). Thus, the TRN is ideally located for modulating thalamocortical communication via neocortical driven feed-forward or thalamic driven feed-back inhibition.

Ultimately, the magnitude of inhibition onto the dorsal thalamus depends critically on the voltage-state of the TRN neuron. Like most thalamic neurons, TRN neurons respond to excitatory input by discharging Na⁺-dependent action potentials in one of two distinct modes: tonic or burst (Avanzini et al., 1989; Contreras et al., 1993; Llinas and Jahnsen, 1982; Spreafico et al., 1988). Transitioning between burst and tonic mode depends on the presence of the low-threshold transient calcium (Ca²⁺) current, Iₜ, which is inactivated at relatively depolarized membrane potentials and de-inactivated at relatively hyperpolarized membrane potentials (Huguenard, 1996; Huguenard and Prince, 1992). In the de-inactivated state, sufficient excitatory input can activate Iₜ resulting in a large Ca²⁺ depolarization known as a low threshold spike (LTS), which in turn generates a high frequency burst discharge of Na⁺-dependent action potentials.
Despite understanding the role of I_T in generating burst output, how this current is distributed across the neuron and more importantly how it contributes to the integration of afferent information remains speculative. Previous studies have suggested a dendritic distribution of I_T in TRN neurons, but these studies have been limited due to technical considerations (Cueni et al., 2008; Destexhe et al., 1996; Joksovic et al., 2005; Kovacs et al., 2010). Although this issue has been explored in thalamocortical relay neurons in which there appears to be a heterogeneous distribution of I_T (Destexhe et al., 1998; Munsch et al., 1997; Williams and Stuart, 2000; Zhou et al., 1997), any comparison between TRN and thalamocortical neurons is difficult because of their distinct morphologies and burst properties (Huguenard and Prince, 1992).

In this study we used two-photon laser scanning microscopy (2PLSM) coupled with whole-cell patch recordings to characterize the somatodendritic distribution of I_T along individual TRN dendrites. Here we show that a somatic burst discharge produces a transient Ca^{2+} current along the entire length of TRN dendrites, which increases in magnitude at greater distances from the soma. Importantly, we also show that the direct stimulation of individual distal dendrites can locally activate distal I_T leading to the amplification of afferent input. These finding have important implications for understanding how TRN neuron modulate thalamic transmission, as well as thalamocortical operations.
2.2.0. Methods and materials

2.2.1. Slice preparation

Thalamic slices were prepared from young Sprague-Dawley rats (postnatal age: 11-23 days) of either sex by using published procedures with minor modifications (Govindaiah and Cox, 2004). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg) and perfused with cold oxygenated slicing solution before being decapitated. Brains were quickly removed and placed in cold (4º C) oxygenated (5% CO₂, 95% O₂) slicing solution containing (in mM): 2.5 KCl, 1.25 NaH₂PO₄, 10.0 MgSO₄, 0.5 CaCl₂, 26.0 NaHCO₃, 10.0 glucose, and 234.0 sucrose. Thalamic slices (250-300 µm) were cut in the horizontal plane for TRN/VB recordings or the coronal plane for dLGN recordings. Slices were immediately transferred to a holding chamber with oxygenated physiological saline (31±1º C) containing in mM: 126.0 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 MgCl₂, 2.0 CaCl₂, 26.0 NaHCO₃, and 10.0 glucose. After 15-20 min the holding chamber was reduced to room temperature and slices were further incubated for a minimum of 60 min before recording.

2.2.2. Whole-cell recording procedures

For recording, individual slices were transferred to a recording chamber that was maintained at room temperature (23±2º C) with oxygenated physiological saline (2.5-3 ml/min). Individual neurons were identified using Dodt contrast optics, and whole-cell recordings were obtained using recording pipettes containing (in mM): 117 K-methylsulfonate, 13 KCl, 2.0 MgCl₂, 10.0 HEPES, 2.0 Na₂-ATP and 0.4 Na-GTP (pH 7.3 and 290 mosm). Pipettes also contained a Ca²⁺-sensitive indicator (Fluo-4: 125 µM) and a Ca²⁺-insensitive indicator (Alexa 594: 25 µM). The dual indicators allowed for visualization of small distal dendrites and provided
a substantially less noisy measurement of Ca\textsuperscript{2+} changes than the use of a single indicator (Yasuda et al., 2004). The final pipette solution resulted in a junction potential of approximately 10 mV and was corrected for in all voltage recordings. During recordings, pipette capacitance was neutralized and the access resistance was continually monitored.

Pharmacological agents were prepared and stored as recommended and diluted in physiological saline to a final concentration before use. All antagonists were bath applied 10-15 min before subsequent experimental tests. TTX, ω-conotoxin-MVIIC, 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 6, 7-dinitroquinoxaline-2,3-dione (DNQX), and mibefradil were all purchased from Tocris (Ellisville, MO), and all other compounds from Sigma (St. Louis, MO).

Stimulation of distal dendrites was accomplished by local glutamate application or electrical stimulation. Focal glutamate application was achieved by pressure ejection (0.5-1 mM, 0.75-3 psi, 30-50 ms duration) via a glass pipette (1-3 µm tip diameter). Pipettes also contained Alexa 594 (12.5 µM), to aid in locating, positioning, and visualizing the ejection site. Only focal ejections with a minimal radial spread (<25 µm) were used for analysis. Linescans were performed at the edge of the stimulated area to limit the amount of dendritic movement caused by pressure ejection. Synaptic stimulation was achieved by placing a bipolar electrode adjacent to TRN within the internal capsule (Single stimulus: 40-300 µA, 500 µs duration, 0.1 Hz).

2.2.3. Calcium imaging and analysis

Ca\textsuperscript{2+} imaging was performed simultaneously with whole-cell recordings using a custom two-photon laser scanning microscope (2PLSM, Ultima, Prairie Technologies, Middleton, WI) coupled with a Ti-sapphire laser (MaiTai HP, Spectra Physics, Mountain View, CA). Imaging
was accomplished by laser excitation (820 nm) via a high NA water-immersion objective (60x; Olympus). To allow sufficient filling of dendritic arbor with fluorescent indicators, imaging was initiated 20-30 minutes after obtaining whole cell configuration. Once a location was selected for imaging the fluorescence was averaged over the spatial extent of the structure using linescans (15-20X optical zoom) with a temporal resolution of ~2.5 ms. On average 5 linescans were collected at each location with 10-15 sec intervals between scans.

Image acquisition was performed using custom Prairie View Software and analyzed post-hoc using ImageJ software (NIH, Bethesda, MD). The reported change in fluorescence (ΔG/R) was calculated as the change in fluorescence from baseline of the Ca^{2+}-sensitive indicator (Fluo-4) normalized to the average fluorescence of the Ca^{2+}-insensitive indicator (Alexa 594): ΔG/R = (G_{peak} – G_o) / R_{Avg} (Sabatini et al., 2002). Baseline fluorescence (G_o) was calculated as the average fluorescence of the 200 ms prior to the stimulus. The peak response was calculated as the combined average of each linescan smoothed with a ~30 ms running average. Physiological recordings were analyzed using pClamp software (Molecular Devices, Inc.). Population data are expressed as mean ± standard deviation (SD) and significance was defined as p < 0.05. A paired t-test was used for analyses unless otherwise indicated.
2.3.0. Results

Combining whole-cell electrophysiological recordings with 2PLSM we initially measured dendritic Ca\(^{2+}\) changes in response to a somatic burst discharge in TRN neurons. Individual neurons were loaded via patch pipette with a Ca\(^{2+}\)-insensitive indicator (Alexa 594: 25 μM) to facilitate the localization of small dendrites and a Ca\(^{2+}\)-sensitive indicator (Fluo-4: 125 μM) to monitor Ca\(^{2+}\) changes (Fig. 5A). A single burst discharge was evoked by somatic current injection (25-75 ms; 100-400 pA) while the initial membrane potential at the soma was held between -80 and -90 mV (Fig. 5B). The resulting change in Ca\(^{2+}\) was monitored simultaneously using a high-resolution linescan and quantified as the change in green fluorescence normalized to the average red fluorescence (ΔG/R) (Fig. 5C, D). Although non-bursting neurons have been reported within the TRN (Brunton and Charpak, 1997; Contreras et al., 1992; Lee et al., 2007), in this study all recordings were obtained from neurons that produced burst discharge.

2.3.1. Burst discharge evokes a robust distal Ca\(^{2+}\) response

Consistent with a previous report (Cueni et al., 2008), somatic burst discharge in TRN neurons reliably produced a dendritic Ca\(^{2+}\) response. To determine if the magnitude of the response was dependent on dendritic location, we initially compared proximal and distal Ca\(^{2+}\) responses obtained from the same dendrite (Fig. 6A). In 13 of 14 TRN dendrites examined (93%), a somatic burst discharge produced a larger Ca\(^{2+}\) response at the distal location (>150 μm) when compared to the proximal location (15-25 μm) (Proximal: 20.0±5.6%; Distal: 28.5±7.5%, n = 14 dendrites (D)/12 neurons (N), p<0.001, paired t-test). There were only two instances where two dendrites were sampled from the same neuron. In both cases the pair of dendrites displayed a comparable proximal-distal increase. On average the distal location had a
143% larger response than the proximal location. To determine if the magnitude of the Ca$^{2+}$ response observed in TRN neurons was common to other bursting thalamic cell types we performed the same experiment on thalamocortical projection neurons of the dorsal lateral geniculate nucleus (dLGN) and ventrobasal (VB) nucleus (Fig. 6B, C). For dLGN dendrites, no significant difference was observed between distal (>100 µm) and proximal Ca$^{2+}$ responses (Proximal: 8.0±3.6%, Distal: 7.6±3.5%, n = 7D/5N, p=0.69, paired t-test). In contrast, VB dendrites did generate a larger distal Ca$^{2+}$ response (Proximal: 5.7±3.1%; Distal: 11.0±3.0%, n = 7D/4N, p < 0.01, paired t-test). Although a single burst discharge produced a dendritic Ca$^{2+}$ response in all three thalamic cell types examined, the magnitude of the TRN responses was considerably larger than those of thalamocortical projection neurons.

To further characterize the dendritic/Ca$^{2+}$ relationship in TRN neurons we proceeded to image 22 new dendrites, however, this time we performed multiple linescans (4-6) along the somatodendritic axis at approximately 50 µm intervals (Fig. 7A, B). Of the dendrites examined, 19 (86%) displayed a positive dendritic slope or an increased Ca$^{2+}$ response with a greater measured distance from soma. In general, TRN dendrites displayed a range of slopes with an average increase of 0.05% ΔG/R per µm (maximum: 0.14% per µm; minimum: -0.06% per µm). For most dendrites (68%), the largest Ca$^{2+}$ response was at a distance >100 µm from soma; however, in a small population of dendrites the largest response occurred between 50-75 µm (27%). Despite some variability, the vast majority of TRN dendrites did display an increasing proximal to distal gradient, further supporting the hypothesis that distal locations have larger Ca$^{2+}$ signals.

Considering the relationship between the Ca$^{2+}$ amplitude and dendritic distance (R = 0.34, p<0.01, Pearson correlation, data not shown), could a change in dendritic diameter account for
the change in magnitude? In layer V pyramidal neurons it has been shown that differences in dendritic diameter (i.e., surface-to-volume ratio) can have a significant influence on the amplitude of the Ca\(^{2+}\) response, with smaller/thinner dendrites producing greater changes (Holthoff et al., 2002). When we examined TRN dendrites the diameter did become smaller with increasing distance from soma (Fig. 7C) (R = -0.53, p<0.001, Pearson correlation). However, the relationship between the peak Ca\(^{2+}\) response and dendritic diameter was weak (R = -0.21, p=0.05, Pearson correlation). This suggests that the smaller dendritic size does not completely account for the larger response observed in distal TRN dendrites. More importantly, this also implies that the density of current across the dendritic tree is not evenly distributed (i.e., higher densities at more distal locations).

Like that of TRN neurons, thalamocortical dendrites also became smaller with increased distance from soma (Fig. 7D) (dLGN: R = -0.66, p<0.001; VB: R = -0.73, p<0.001, Pearson correlation). However, unlike TRN neurons VB dendrites had a 3-fold stronger inverse relationship between peak Ca\(^{2+}\) response and dendritic diameter (R = -0.67, p<0.001, Pearson correlation). This argues that the VB proximal/distal differences (Fig. 6C) are primarily due to smaller dendritic size and not a larger current density. No significant correlation between peak Ca\(^{2+}\) response and dendritic diameter was found for dLGN dendrites (R = -0.34, p=0.054, Pearson correlation). These analyses indicate that the Ca\(^{2+}\) distribution in TRN dendrites are fundamentally different from those observed in thalamocortical projection neurons.

For a more quantitative comparison within and across thalamic cell types, we divided dendritic length into five subgroups based on radial distance from soma: soma (0 \(\mu\)m), proximal (15-25 \(\mu\)m), intermediate (50-75 \(\mu\)m), distal I (100-130 \(\mu\)m), and distal II (>150 \(\mu\)m; Fig. 7E). For TRN neurons, the average Ca\(^{2+}\) response for each dendritic region was significantly larger
than soma (\(p<0.001\), one-way ANOVA with a post-hoc Bonferroni Test) (TRN Soma: 5.8±1.6% \(\Delta G/R\), \(n = 17N\); Proximal: 19.5±4.9%, \(n = 22D/17N\); Intermediate: 25.2±8.9%, \(n = 22D/17N\); Distal I: 26.7±7.0%, \(n = 22D/17N\); Distal II: 28.5±7.5%, \(n = 14D/14N\)). In addition to the somatic/dendritic difference, the \(\text{Ca}^{2+}\) responses taken from both distal I and distal II locations were significantly larger than proximal (\(p<0.01\), one-way ANOVA with a post-hoc Bonferroni Test). Compared to thalamocortical neurons, TRN neurons had significantly larger \(\text{Ca}^{2+}\) responses than both dLGN and VB neurons (\(p < 0.001\), two-way ANOVA with a post-hoc Bonferroni Test).

### 2.3.2. Dendritic \(\text{Ca}^{2+}\) responses are dependent on firing mode

In many neurons, action potentials initiated in either the axon or soma can backpropagate into the dendritic arbor (Stuart et al., 1997; Stuart and Sakmann, 1994). In pyramidal neurons of the neocortex and hippocampus, backpropagating action potentials have been shown to produce \(\text{Ca}^{2+}\) responses along their dendrites (Jaffe et al., 1992; Magee and Johnston, 1997; Markram et al., 1995; Schiller et al., 1995). To characterize if the observed \(\text{Ca}^{2+}\) responses were possibly evoked by backpropagating action potentials in TRN neurons, we elicited either a single action potential (AP) or a tonic burst of action potentials (4 APs at 100 Hz) using brief somatic current injections (Fig. 8A). To ensure TRN neurons were not in burst mode (i.e., deinactivation of \(I_T\)), the membrane potential of the soma was adjusted with current to -60 mV. Under these conditions a single and tonic burst of APs produced a small \(\text{Ca}^{2+}\) response at proximal dendritic locations (Fig. 8B; Single AP: 3.2±2.0% \(\Delta G/R\), \(n = 6\); Tonic burst: 6.1±2.0% \(\Delta G/R\), \(n = 6\)). At more distal locations, the \(\text{Ca}^{2+}\) responses were difficult to detect from baseline (Single AP: 2.3±1.0% \(\Delta G/R\), \(n = 5\); Tonic Burst: 2.6±0.9% \(\Delta G/R\), \(n = 5\)). Overall, the dendritic \(\text{Ca}^{2+}\) responses evoked by
action potential discharge at -60 mV were significantly smaller than those produced via a burst discharge at -80 mV (Fig. 8C; p<0.001, two-way ANOVA). These data strongly suggest that an underlying LTS must be responsible for the dendritic Ca\(^{2+}\) response.

### 2.3.3. Dendritic Ca\(^{2+}\) responses are mediated by \(I_T\)

To determine the nature of the dendritic Ca\(^{2+}\) response in TRN neurons, we initially tested if the burst-induced response was dependent on the activation of voltage-gated Na\(^+\)-channels. Using tetrodotoxin (TTX: 0.5 µM) to block voltage-gated Na\(^+\)channels, the same somatic current injection revealed a transient, Ca\(^{2+}\)-dependent LTS (Fig. 9A). The LTS in turn produced a Ca\(^{2+}\) response along the entire length of the dendrite similar to that generated by a burst discharge (p=0.84, two-way ANOVA). To further confirm the independence from voltage-gated Na\(^+\)-channels, distal (>150 µm) responses were measured from the same location before and after TTX application. Under these conditions, the magnitude of the response remained the same (Fig. 9B: 117.4±34.0% of control, n=15D/15N, p=0.17, paired t-test). These results indicate that for TRN neurons the magnitude of the burst-induced dendritic Ca\(^{2+}\) response is independent of both the backpropagation of somatic action potentials and dendritic voltage-gated Na\(^+\)-channels.

Focusing on the distal dendrites (>150 µm), we next determined which type(s) of voltage-gated Ca\(^{2+}\) channels underlie the somatic evoked distal response. While in the presence of TTX (0.5 µM), the addition of the L-type channel blocker nifedipine (NIF: 1, 10 µM) reduced the LTS amplitude recorded at the soma (1µM: ~6% reduction; 10µM: ~10% reduction), but did not alter the magnitude of the distal Ca\(^{2+}\) response (Fig. 9C, D: 1.0 µM: 106±16% of control, n = 4D/4N, p=0.5; 10 µM: 107±22.1% of control, n = 5D/5N, p=0.7, paired t-test). Subsequent
addition of the T-type channel blocker mibefradil (MIB: 40-50 µM) significantly reduced the LTS amplitude recorded at the soma (~39% reduction), and nearly eliminated the distal Ca\(^{2+}\) response (MIB: 85.5±6.1% reduction, n = 5D/5N, p<0.01, paired t-test). To confirm that the mibefradil block was not affected by nifedipine, we also tested mibefradil alone and observed a similar reduction (MIB alone: 97.5%±1.6% reduction, n = 4D/4N, p<0.001, paired t-test). Mibefradil also blocked the Ca\(^{2+}\) responses at all other dendritic locations (compared to control condition, p<0.001, two-way ANOVA). Similar to nifedipine, the N,P/Q-type Ca\(^{2+}\) channel blocker ω-conotoxin-MVIIC (20-40 nM) did not significantly alter the dendritic Ca\(^{2+}\) response (114±21.2% of control, n = 5D/5N, p=0.5, paired t-test). Together, these results suggest that the LTS/burst evoked Ca\(^{2+}\) response in TRN dendrites is strongly mediated by the activation of T-type Ca\(^{2+}\) channels.

2.3.4. Distal IT is activated independent of soma/proximal dendrites

Although a distal Ca\(^{2+}\) response was always produced with a somatic evoked LTS/burst, it remained unclear whether this response was generated by distally located T-type Ca\(^{2+}\) channels or was simply the result of the passive invasion of soma/proximal signals into the dendrites. To address this issue directly, individual distal TRN dendrites were stimulated with brief, focal glutamate application (0.5-1.0 mM; 30-50 ms duration) via pressure ejection (Fig. 10A). In control conditions (ACSF) and holding the soma at a hyperpolarized potential (~80 mV), glutamate application to an individual distal dendrite (>125 µm) generated a strong burst discharge recorded at the soma (Fig. 10B). Following the addition of TTX (0.5 µM), the same application of glutamate now produced an LTS (Fig. 10B, TTX). No discernable difference was detected between the glutamate evoked burst/LTS and those generated via somatic current
injection (APs per burst: soma current injection: 3.1±2.1 APs, glutamate: 3.2±2.0 APs, n = 9, p=0.25, paired t-test; LTS amplitude: soma current injection: 43.1±5.8 mV, glutamate: 43.9±4.9 mV, n = 9, p=0.30, paired t-test). When the soma was depolarized to inactivate soma/proximal I\textsubscript{T} (-60 mV), distal glutamate application resulted in a much smaller, EPSP-like depolarization (Fig. 10B: -80mV: 44.9±4.2 mV; -60mV: 11.0±4.4 mV, n = 8, p<0.001, paired t-test). These data suggest that distal afferent signals are capable, when the appropriate voltage-dependent conditions are present at the soma, of generating a burst/LTS output from the neuron.

When examining the glutamate-evoked Ca\textsuperscript{2+} response, there was a clear relationship between the response magnitude and the success/failure of generating a somatic LTS (Fig. 10C). When a subthreshold application of glutamate was ejected it produced a small depolarization at the soma with little or no distal Ca\textsuperscript{2+} response (Fig. 10C, red traces); however, a large dendritic response was always associated with a suprathreshold application of glutamate which in turn always produced a LTS at the soma (Fig. 10C, black traces). More importantly, regardless of whether the somatic membrane potential was held at a hyperpolarized (-80 mV) or depolarized (-60 mV) level, suprathreshold glutamate application always produced a robust Ca\textsuperscript{2+} response in the distal dendrites (Fig. 10D). Compared to the Ca\textsuperscript{2+} response elicited by a somatic current injection, the glutamate-evoked response was prolonged. The amplitude of the glutamate-evoked response at -80 mV (24.0±7.0% ΔG/R) was larger than both the somatic evoked (19.8±9.1% ΔG/R, p<0.05, paired t-test) and the glutamate-evoked response at -60 mV (18.3±6.3% ΔG/R, n =12, p<0.001, paired t-test, Fig. 10E). No significant difference was found between the somatic evoked and glutamate-evoked response at -60 mV (p=0.34, paired t-test). These results indicate that focal glutamate application to a distal dendrite is sufficient to activate a large distal Ca\textsuperscript{2+} response independent of soma/proximal dendrites.
Considering glutamate stimulation could activate Ca\(^{2+}\) permeable N-methyl-D-aspartic acid (NMDA) receptors, we tested the antagonist CPP on the glutamate-evoked distal Ca\(^{2+}\) response. Bath application of CPP (10-20 µM) produced a small, but statistically significant attenuation of the glutamate-evoked Ca\(^{2+}\) response (Fig. 11A, Glu-Control: 36.9±6.6% ΔG/R, Glu-TTX+CPP: 31.6±5.8% ΔG/R, n = 8, p<0.05, paired t-test). Importantly, the glutamate-evoked Ca\(^{2+}\) response in the presence of CPP did not differ from the response evoked by somatic current injection (30.2±8.2% ΔG/R; n = 8, p=0.50, paired t-test). In all eight neurons tested, CPP also reduced the prolonged Ca\(^{2+}\) response observed with glutamate application. The subsequent application of the AMPA receptor antagonist DNQX (20-40 µM) eliminated the glutamate-evoked Ca\(^{2+}\) response (CPP+DNQX: 5.0±5.8% ΔG/R; n=3). CPP nor CPP+DNQX altered the dendritic Ca\(^{2+}\) responses evoked by somatic current injection (p=0.92, n = 7, paired t-test and p=0.93, n=6, paired t-test, respectively).

We next tested the contribution of T-type Ca\(^{2+}\) channels on the glutamate-evoked Ca\(^{2+}\) response (Fig. 11B). In mibefradil (50 µM), the amplitude of the glutamate-evoked dendritic Ca\(^{2+}\) responses were significantly reduced regardless of the somatic membrane potential (@-80 mV: Control: 25.0±6.6% ΔG/R, n = 5; +MIB: 6.3±2.6% ΔG/R, n = 5, p<0.01, paired t-test; @-60 mV: Control: 19.5±5.9% ΔG/R, n = 4; +MIB: 7.7±4.8% ΔG/R, n = 4, p<0.01, paired t-test). These findings suggest that distal glutamate application to an individual TRN dendrite is sufficient to activate local T-type Ca\(^{2+}\) channels. Considering that mibefradil reduced both the distal Ca\(^{2+}\) response and corresponding somatic depolarization (58% and 12% reduction at -80 mV and -60 mV, respectively), it is likely that I\(_T\) is amplifying afferent input.
2.3.5. Synaptic activation of distal $I_T$

To probe the functional relevance of distal $I_T$ amplification, we stimulated local excitatory synaptic afferents in place of glutamate application. For these experiments the stimulating electrode was positioned lateral to the TRN within the internal capsule so to activate both corticothalamic and thalamocortical fibers (Golshani et al., 2001; Zhang and Jones, 2004). Using a single stimulus (40-300 µA, 500 µs, 0.1 Hz), burst discharge was generated in our somatic recording when the neuron was held at -80 mV, along with a corresponding large distal Ca$^{2+}$ response (Fig. 12A). The magnitude of the synaptically evoked distal Ca$^{2+}$ response did not differ from the response evoked by somatic current injection (@-80 mV: Synaptic: 27.6±4.0% ΔG/R; Soma current injection: 23.7±6.9% ΔG/R, n = 4, p=0.23, paired t-test). Subsequent application of CPP (20 µM) did not significantly alter the amplitude of the synaptically-evoked Ca$^{2+}$ response (+CPP: 23.1±10.7% ΔG/R, n = 4, p=0.28, paired t-test); however, addition of DNQX (40 µM) eliminated the response (CPP+DNQX: 2.0±0.4% ΔG/R, n = 4, p<0.001, paired t-test). When the soma was depolarized to -60 mV, the same stimulus generated 1-2 action potentials (Fig. 12B). Under these conditions the amplitude of the synaptically evoked Ca$^{2+}$ response displayed a considerable degree of variability (45.9% CV, n = 4); however the large responses generated (21.1±8.3% ΔG/R, n = 4) were not statistically different from the responses generated by a somatic induced LTS (23.7±6.9% ΔG/R, p=0.48, paired t-test). Subsequent bath application of CPP (20 µM) did not alter the amplitude of the Ca$^{2+}$ response (+CPP: 17.2±12.7% ΔG/R, n = 4, p=0.36, paired t-test), whereas DNQX eliminated response (1.5±0.5% ΔG/R, n = 4, p<0.02, paired t-test).
2.4.0. Discussion

The results of the present study indicate that a somatic burst discharge or activation of an LTS can evoke a robust Ca\(^{2+}\) response in TRN dendrites mediated by \(I_T\). \(Ca^{2+}\) influx was observed along the entire length of the dendrite with the largest response typically occurring at more distal locations (>100 µm). Although all thalamic neurons tested produced a change in dendritic \(Ca^{2+}\) in response to burst discharge, the magnitude within TRN dendrites was significantly larger than those observed in thalamocortical relay neurons. In addition, we show that distally located \(I_T\) can be activated independently from the soma/proximal dendrites, therefore possibly serving to boost local afferent inputs onto TRN neurons. The activation of distal \(I_T\), by either focal glutamate application or synaptic activation, required activation of AMPA-, but not NMDA-glutamate receptors. Since both \(I_T\) kinetics and amplitude are highly temperature dependent (Coulter et al., 1989; Takahashi et al., 1991), we feel performing these experiments at room temperature would have altered the typical physiological response but may have provided a more accurate measurement of the peak change (Markram et al., 1995). Overall these findings provide the first physiological evidence of the somatodendritic distribution of \(I_T\) in TRN neurons, as well as insight regarding the potential functional role of \(I_T\) in synaptic integration within reticular neurons.

2.4.1. Somatodendritic distribution of \(I_T\) in TRN neurons

It is well documented that activation of \(I_T\) and the resulting LTS are responsible for generating a burst discharge of action potentials in TRN and thalamocortical relay neurons (Avanzini et al., 1989; Coulter et al., 1989; Crunelli et al., 1989; Deschenes et al., 1984; Domich et al., 1986; Hernandez-Cruz and Pape, 1989; Huguenard and Prince, 1992; Jahnsen and Llinas,
However, data regarding the dendritic distribution of \( I_T \) in the thalamus has been limited to studies focusing on thalamocortical relay neurons (Destexhe et al., 1998; Munsch et al., 1997; Williams and Stuart, 2000; Zhou et al., 1997). For the TRN neuron, any accurate physiological quantification of the distribution of \( I_T \) has been lacking. The results of the present study indicate a heterogeneous dendritic distribution of \( I_T \) in TRN neurons, results which are consistent with an earlier computational study (Destexhe et al., 1996). Although an accurate quantification of T-type channel density is difficult to extrapolate, the weak correlation between \( \text{Ca}^{2+} \) response amplitude and dendritic diameter implies an increased density at more distal locations (Fig. 7C). This interpretation corresponds well with a recent anatomical study which found a higher density of T-type \( \text{Ca}^{2+} \) channels in small-diameter TRN dendrites, likely representing distal dendrites (Kovacs et al., 2010).

### 2.4.2. State-dependent amplification

Our findings also support the general hypothesis that \( I_T \) may serve to amplify afferent input as suggested in different neuronal types (Gillessen and Alzheimer, 1997; Goldberg et al., 2004; Magee et al., 1995; Urban et al., 1998; Watanabe et al., 1998; Williams and Stuart, 2000; Williams et al., 1997). Considering the voltage dependence of \( I_T \), namely being activated from relatively hyperpolarized potentials, a non-uniform membrane potential along the somatodendritic axis could have significant influence on dendritic excitability, synaptic amplification, and ultimately action potential output. For example, if the somatodendritic axis is uniformly hyperpolarized, amplified distal input would be uninterrupted and actively propagated toward the soma, ultimately producing a burst discharge. Conversely, if the soma-proximal
dendrites were depolarized relative to the distal dendrites, distal signals would still be amplified considering activation of \( I_T \), resulting in a larger afferent EPSP at the soma level but not producing a burst discharge. In the later scenario, synapses located further from the soma would still have an ability to contribute to somatic output given sufficient activation of distal \( I_T \). Finally, different neuromodulators thought to depolarize TRN via actions at distal dendrites (e.g. cholecystokinin, glutamate via metabotropic receptors) could inactivate \( I_T \), and thereby dampen afferent inputs by attenuating the amplification by \( I_T \) (Cox et al., 1995; Sohal et al., 1998). Thus, at the dendritic level, \( I_T \) could serve to regulate the spatial integrative properties of the TRN neuron and interactions between distinct dendritic compartments (i.e., proximal/soma and distal).

Given TRN neurons can have multiple primary dendritic branches (Jones, 1975; Lubke, 1993; Ohara and Havton, 1996; Scheibel and Scheibel, 1966) an interesting and potentially important issue is whether or not the voltage-state across the somatodendritic axis could differ between individual dendrites of the same TRN neuron. Such dendritic isolation could promote highly localized processing and would allow individual branches to act as independent integrators of different information (Euler et al., 2002; Hausser and Mel, 2003).

### 2.4.3. Heterogeneity amongst TRN neurons

Although this study focused on the functional role of dendritic \( I_T \) in TRN neurons capable of burst discharge, there are studies which have clearly identified a sub-population of non-bursting TRN neurons (Brunton and Charpak, 1997; Contreras et al., 1992; Lee et al., 2007). For these neurons, the lack of burst discharge seems to result from a minimal amount of \( I_T \) recorded at the soma. However, the inability to induce a somatic burst discharge does not suggest a complete absences of dendritic \( I_T \). It is possible that non-bursting TRN neurons could represent a
sub-population with an extreme proximal-distal difference in their distribution of \( I_T \). In theory, maintaining a high density of distal \( I_T \) would still allow for the amplification of distal afferent input with the lack of soma/proximal \( I_T \) preventing the neuron from generating a burst response. In fact, a more distal distribution of \( I_T \) in non-bursting TRN neurons is supported by the fact that in a subset of these neurons a small LTS could be unmasked when a \( K^+ \)-channel blocker was applied (Lee et al., 2007). Although such a somatodendritic distribution remains to be tested, the functional consequence of such a distribution may play an important role in the transfer of modality-specific information as well as thalamocortical operations.

### 2.4.4. Functional significance of distal \( I_T \)

Considering the functional architecture of the TRN, elongated nature of TRN dendrites, and robust responses in distal processes, could distal dendrites serve as sites of intense integration within and across different modalities? The TRN is situated at the interface between the thalamus and neocortex, and receives collateral innervation from both thalamocortical and corticothalamic projection neurons. As the fibers pass through the TRN, they assemble in several well-defined modality-related sectors (Guillery and Harting, 2003; Jones, 1975; Montero, 1997). Furthermore, sectors can be subdivided into slabs that receive afferent information from focal areas of the neocortex and dorsal thalamus or slabs of TRN neurons projecting to specific regions of the dorsal thalamus (Crabtree, 1999).

Since most TRN neurons possess elongated dendrites (Jones, 1975; Lubke, 1993; Ohara and Havton, 1996; Scheibel and Scheibel, 1966), it is likely that neurons near the borders would have some distal dendrites cross into neighboring sectors or slabs. Since the majority of afferent inputs onto distal dendrites arise from corticothalamic neurons (Liu et al., 1995), it is possible
that a strong “top-down” modulation of distal TRN dendrites would significantly influence the integrative properties of the TRN by altering the resting voltage-state of the somatodendritic axis. Such “top-down” modulation could arise from one or a combination of inputs originating from either the primary sensory neocortex (Montero, 2000) or prefrontal cortex (Zikopoulos and Barbas, 2006). Ultimately, the degree of “top-down” influence onto distal dendrites could dictate the degree in which the TRN gates “bottom-up” information transfer within and/or across different modalities. It is also possible that dendritic I\textsubscript{T} could serve to facilitate intra-TRN communication. The fact that many TRN neurons are coupled by electrical synapses (Landisman and Connors, 2005; Landisman et al., 2002; Long et al., 2004) and the electrical signals through these synapses are subjected to severe attenuation and low-pass filtering (Galarreta and Hestrin, 2001), it is possible that high densities of distal I\textsubscript{T} could also serve to amplify dendritic electrical synapses among TRN neurons. It is also intriguing to speculate as to whether I\textsubscript{T} and electrical synapses could help generate and preserve the endogenous spindle rhythmicity observed in the TRN \textit{in vivo} (Steriade et al., 1987). The fact that we did not observe strong distal Ca\textsuperscript{2+} responses in relay neurons and they are not electrically coupled in the relatively mature animal (Lee et al., 2010) further supports this possibility.

To date there are limited reports of \textit{in vivo} electrophysiological recordings of TRN neurons responding to more than one sensory modality (Shosaku and Sumitomo, 1983; Sugitani, 1979). However, recent studies indicate that TRN activity is subjected to cross-modal modulation (Yu et al., 2009) and may be affected by various attentional demands (McAlonan et al., 2006, 2008; Yu et al., 2009). In conjunction with our results, we speculate that the contribution of distal dendrites may underlie such cross-modal actions in a state-dependent
manner. Clearly, the resting state of the dendrites and distribution of $I_T$ will have significant influence on thalamocortical operations that requires future investigation.
2.5.0. Figures

Figure 5: Measurement of dendritic Ca$^{2+}$ responses evoked by somatic burst discharge in TRN neurons. A, Stacked 2PLSM image of a TRN neuron filled. The yellow box indicates the dendritic region from which a linescan was performed (75 µm). B, Somatic recording of a burst discharge elicited from a hyperpolarized holding potential by injecting a short depolarizing current pulse. C, Simultaneous linescan of both the calcium-insensitive (Alexa 594: Red) and calcium-sensitive (Fluo-4: Green) fluorescent signals. D, The average ΔG/R response obtained from the dendritic region shown in A. The average (dark green line) and standard deviation (light green envelope) were obtained from 5 consecutive burst discharges.
Figure 6: Burst discharge produces a robust Ca\(^{2+}\) response in distal dendrites of TRN neurons. **A**, Left, stacked 2PLSM image of an example TRN neuron filled. The blue box indicates the dendritic region from which a proximal linescan was typically obtained (15-25 µm). The red box indicates the dendritic region from which a distal linescan was typically obtained (>150 µm). Middle, Proximal (blue) and distal (red) Ca\(^{2+}\) responses obtained from a different TRN neuron. Right, Population data collected from 14 dendrites. **B**, Left, stacked 2PLSM image of an example dLGN neuron filled. Blue box indicates dendritic region from which a proximal linescan was typically obtained (15-25 µm). The red box indicates the dendritic region from which a distal linescan was typically obtained (>100 µm). Middle, Proximal (blue) and distal (red) Ca\(^{2+}\) responses obtained from a different dLGN neuron. Right, Population data collected from 7 dendrites. **C**, Left, stacked 2PLSM image of an example VB neuron filled. Boxes represent the same region as in **B**. Middle, Proximal (blue) and distal (red) Ca\(^{2+}\) responses obtained from a different VB neuron. Right, Population data collected from 7 dendrites.
Figure 7: The TRN dendritic/Ca\(^{2+}\) relationship is independent of dendritic size. A, Top, stacked 2PLSM image of an example TRN neuron filled. Boxes indicate where linescans were performed. Bottom, corresponding Ca\(^{2+}\) responses obtained from the neuron above. B, Left, plot depicts Ca\(^{2+}\) responses obtained along 14 different TRN dendrites as a function of dendritic distance. C, Left, correlation between TRN dendrite diameter and measured distance from soma. Right, correlation between the peak Ca\(^{2+}\) response and the measured TRN dendritic diameter. D, Left, correlation between dendrite diameter and measured distance from soma for both dLGN (blue) and VB neurons (red). Right, correlation between Ca\(^{2+}\) response and the measured dendritic diameter for both thalamocortical projection neurons. E, Left, Diagram illustrating how data were pooled based on their radial distance from soma (soma, proximal, intermediate, distal I, and distal II). Right, Plot summarizing the average response for the three different thalamic cell types (dLGN: Soma, 2.9±2.5%, n = 7N; Prox., 7.9±4.2%, n = 11D/7N; Inter., 8.6±4.2%, n = 13D/8N; Dist.1, 8.1±3.2%, n = 9D/6N; VB: Soma, 2.4±1.0%, n = 4N; Prox., 5.9±2.9%, n = 8D/4N; Inter., 10.2±2.7%, n = 8D/4N; Dist.1, 11.0±3.0%, n = 7D/4N).
Figure 8: Ca^{2+} responses are dependent on firing mode. A, Action potentials were discharged in one of three ways. Burst discharge (black) was evoked as previously described in Fig. 5B. A single (blue) or tonic burst (red) of action potentials were evoked by holding the soma near -60 mV and injecting short (5 ms) depolarizing current pulses. A tonic burst consisted of 4 pulses delivered at 100 Hz. B, Ca^{2+} responses for each of the conditions shown in A at different distances obtained from the same dendrite. The responses shown are from the same dendrite. C, plot summarizing the average response to burst discharge (black), tonic burst (red) and single action potential (blue). For reference the average response to a burst discharge is shown in black.
Figure 9: TRN dendritic Ca\textsuperscript{2+} are independent of voltage-gated Na\textsuperscript{+} channels but are strongly dependent on voltage-gated T-type Ca\textsuperscript{2+} channels. A, Left, Example of a somatic recording from a TRN neuron in which a depolarizing current step evoked a burst response in control conditions (ACSF), and a LTS following TTX application (red trace). Right, Population graph illustrating the Ca\textsuperscript{2+} responses recorded at different dendritic locations produced by somatic current injection in control conditions (ACSF, black) and in TTX (red). The Ca\textsuperscript{2+} responses in TTX: Soma, 5.3±0.7%, n = 4N; Prox., 19.2±5.8%, n = 5D/4N; Inter., 24.1±6.5%, n = 5D/4N; Dist.1, 27.4±2.0%, n = 5D/4N; Dist.2, 28.2±1.5%, n = 5D/5N. B, An example of a Ca\textsuperscript{2+} response obtained from the same location before (black) and after TTX (red) application. Top, in TTX, depolarizing current step evokes an LTS (black). Addition of nifedipine (NIF: 10 µM) slightly alters the LTS (blue); however, subsequent addition of mibefradil (MIB: 50 µM) strongly attenuates the LTS (green). Bottom, from the same neuron, the control Ca\textsuperscript{2+} response (TTX, black) is slightly changed in the presence of nifedipine (blue), but is almost completely blocked by mibefradil (green). D, Population data for five different distal dendrites (>150 µm) obtained from different TRN neurons. The Ca\textsuperscript{2+} responses are standardized to the response obtained in TTX, and the red data points indicate the mean ± SD.
Figure 10: Focal glutamate application onto a distal dendrite evokes local Ca$^{2+}$ current independent of the soma/proximal dendrites. A, stacked 2PLSM image of a TRN neuron filled and pressure-ejection pipette near a distal dendrite (185 µm). The pipette contained glutamate (0.5-1 mM in ACSF) and Alexa 594 (12.5 µM), the latter aiding in the placement of the pipette and estimate of the spread of glutamate ejection, which is represented by the white-dotted line (~15-20 µm radius). Linescans were performed near the edge of the stimulated area (yellow box). B, Glutamate application (Glu) to distal dendrite produced a burst discharge recorded at the soma in control conditions (ACSF). In TTX (0.5 µM), glutamate produced an LTS when the cell was initially held at -85 mV, but when the cell was depolarized (-60 mV), the same stimulation produced a small transient depolarization (EPSP-like). C, Left, Somatic response to 5 separate glutamate applications with different puff intensities (0.75-3 psi, 50 ms duration). Right, corresponding Ca$^{2+}$ responses recorded near the glutamate pipette. Two subthreshold (red) and three supratreshold (black) responses are shown. D, Examples of Ca$^{2+}$ responses measured at a distal dendrite (175 µm) in the presence of TTX resulting from somatic current injection (CI: black trace) and distal glutamate application. The latter Ca$^{2+}$ responses depict glutamate-evoked responses when the soma was either held at -80 mV (blue) or -60 mV (red). E, The plot illustrates the amplitude of the glutamate-evoked responses in these two conditions (-85 or -60 mV at soma) relative to the Ca$^{2+}$ responses evoked by somatic current injection.
Figure 11: Characterization of glutamate evoked distal Ca\(^{2+}\) signals. A (i), Experimental design consisted of applying glutamate (Glu) to an individual distal TRN dendrite while holding the soma at -80 mV in a bath solution that contained TTX (0.5 µM). A (ii), Glutamate-evoked response recorded at the soma before (Control) and after CPP or CPP+DNQX application. A (iii), Left, in the same cell as A(ii) the corresponding glutamate-evoked Ca\(^{2+}\) response imaged at a distal location. Right, for reference a LTS Ca\(^{2+}\) response evoked by a somatic current injection (CI) is shown. A (iv), average amplitude of the Ca\(^{2+}\) response for each of the four conditions tested are plotted. B (i), Experimental design consisted of applying glutamate (Glu) to an individual distal TRN dendrite while holding the soma at either -80 or -60 mV in a bath solution that contained TTX (0.5 µM). B (ii), Glutamate-evoked response recorded at the soma while holding at -80 mV before (Control) and after mibefradil (MIB; 50 µm) application. B (iii), Control trace (black) is the response to distal glutamate application in presence of TTX (Left: -80 mV; Right: -60 mV). Following the addition of mibefradil, the subsequent Ca\(^{2+}\) responses to glutamate application are significantly reduced. Peak Ca\(^{2+}\) responses for this experiment were measured within the grey box. B (iv), average amplitude of the Ca\(^{2+}\) response for each of the four conditions tested are plotted.
Figure 12: Characterization of synaptic evoked distal Ca\textsuperscript{2+} signals. A (i), Experimental design consisted of stimulating the internal capsule using a single stimulus (Stim) while holding TRN neurons the soma at -80 mV in a control ACSF bath solution. A (ii), Somatic recording of a synaptically evoked burst discharge while holding at -80 mV. A (iii), Left, distal Ca\textsuperscript{2+} response evoked by synaptic stimulation of the internal capsule (black). For reference a control (red) response to a somatic current injection is also shown. Right, resulting synaptically evoked Ca\textsuperscript{2+} responses following the bath application of CPP (green) and CPP+DNQX (blue). A (iv), Average amplitude of the Ca\textsuperscript{2+} response for each of the four conditions tested are plotted. B (i), Experimental design consisted of stimulating the internal capsule using a single stimulus (Stim) while holding TRN neurons the soma at -60 mV in a control ACSF bath solution. B (ii), Somatic recording of a synaptically evoked burst discharge while holding at -60 mV. B (iii), Left, a single Ca\textsuperscript{2+} response (black) evoked by synaptic stimulation of the internal capsule. Subsequent Ca\textsuperscript{2+} responses after bath application of CPP (green) and CPP+DNQX (blue). B (iv), Average amplitude of the Ca\textsuperscript{2+} response for each of the four conditions tested are plotted.
2.6.0. References


Local dendrodendritic inhibition regulates fast synaptic transmission in visual thalamus\(^1\)

3.0.0. Abstract

Inhibition from thalamic interneurons plays a critical role in modulating information transfer between thalamus and neocortex. Interestingly, these neurons yield inhibition via two distinct outputs: presynaptic dendrites that innervate thalamocortical relay neurons and axonal outputs. Since the dendrites of thalamic interneurons are the primary targets of incoming synaptic information, it has been hypothesized that local synaptic input could produce highly focused dendritic output. To gain additional insight to the computational power of these presynaptic dendrites, we have combined two-photon laser scanning microscopy, glutamate uncaging, and whole-cell electrophysiological recordings in order to locally activate dendritic terminals and study their inhibitory contribution onto rat thalamocortical relay neurons. Our findings demonstrate that local dendritic release from thalamic interneurons is controlled locally by AMPA/NMDA receptor-mediated recruitment of L-type calcium channels. Moreover, by mapping these connections with single-dendrite resolution we not only found that presynaptic dendrites preferentially target proximal regions, but such actions differ significantly across branches. Furthermore, local stimulation of interneuron dendrites did not result in global excitation, supporting the notion that these interneurons can operate as multiplexors, containing numerous independently operating input-output devices.

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3.1.0. Introduction

Inhibition plays an essential role in thalamocortical communication. In the visual thalamus, inhibition modulates receptive field properties, signal selectivity, information encoding, and the firing mode of thalamocortical relay neurons (Holdefer et al., 1989; Hubel and Wiesel, 1961; Sillito and Kemp, 1983; Wang et al., 2011; Wang et al., 2007). Local thalamic interneurons provide one of the primary sources of inhibition in the visual thalamus. These neurons are of great interest considering they produce inhibitory output via traditional axonal terminals (termed F1 terminals) as well as specialized dendritic terminals (termed F2 terminals), which serve both pre- and post-synaptic roles (Famiglietti and Peters, 1972; Guillery, 1969; Hamos et al., 1985; Montero, 1986; Ralston, 1971).

Most F2 terminals are presynaptic to a relay neuron dendrite and postsynaptic to an excitatory retinogeniculate terminal that in turn is presynaptic to the same relay neuron dendrite (Hamos et al., 1985; Wilson et al., 1984) (Fig. 13a). However, it is important to note that not all F2 and retinogeniculate terminals participate in this triadic arrangement (Datskovskiaia et al., 2001; Hamos et al., 1987). The unique circuitry of the triad enables retinogeniculate output to produce 1) a monosynaptic excitation of a postsynaptic relay neuron dendrite and 2) a disynaptic inhibition via the F2 terminal. Previous studies indicate that F2 terminal output is regulated by local metabotropic glutamate receptors (mGluRs) (Cox et al., 1998; Godwin et al., 1996; Govindaiah and Cox, 2004; Govindaiah and Cox, 2006). There is also evidence to suggest that activation of ionotropic glutamate receptors (iGluRs) can increase dendritic output (Acuna-Goycolea et al., 2008; Blitz and Regehr, 2005; Cox and Sherman, 2000), however it is unclear if such actions occur local to F2 terminals or independent of axonal output from interneurons.
Since F2 terminals are typically found on distal dendrites of interneurons and are commonly coupled with an excitatory presynaptic terminal, it has been hypothesized that F2 terminals may behave as independent input-output devices (Bloomfield and Sherman, 1989; Cox et al., 1998). The ability to perform localized input-output computations independent of somatic activity would allow thalamic interneurons to behave as multiplexors, capable of transmitting numerous signals through independently operating F2 terminals. Studies have demonstrated that dendritic GABA release can take place independent of activity at the soma/axon (Acuna-Goycolea et al., 2008; Cox and Sherman, 2000; Cox et al., 1998; Govindaiah and Cox, 2006); however, these studies have relied on widespread pharmacological and synaptic stimulation in order to activate F2 terminals. Thus, both approaches result in global activation of the entire dendritic arbor and make it impossible to assay whether F2 terminals produce more localized input-output computations.

Here, we have examined local GABA release from the presynaptic dendrites of thalamic interneurons using local glutamate uncaging. This approach allowed us to excite distinct regions of the dendritic tree and thus mimic the function of presynaptic excitatory terminals (Callaway and Yuste, 2002). Our data show that local dendrodendritic inhibition regulates fast synaptic transmission differentially across the dendritic tree of thalamocortical relay neurons. Moreover, this output appears to occur locally through AMPA/NMDA receptor activation and subsequent recruitment of L-type calcium channels.
3.2.0. Methods and materials

3.2.1. Slice preparation

Thalamic slices were prepared from young Sprague-Dawley rats (postnatal age: 14-23 days) of either sex as previously described (Crandall et al., 2010; Govindaiah et al., 2010). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg) and perfused with cold, oxygenated slicing solution before being decapitated. Once brains were removed they were placed immediately in cold (4° C) oxygenated (5% CO₂, 95% O₂) slicing solution containing (in mM): 2.5 KCl, 1.25 NaH₂PO₄, 10.0 MgSO₄, 0.5 CaCl₂, 26.0 NaHCO₃, 10.0 Glucose, and 234.0 sucrose. Thalamic slices (270-300 µm) were cut in the coronal plane for dorsal lateral geniculate nucleus (dLGN) recordings and the horizontal plane for ventrobasal thalamic nucleus (VB) recordings. After slicing, each tissue section was immediately transferred to a holding chamber with warmed (31±1 °C), oxygenated physiological saline containing in mM: 126.0 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 MgCl₂, 2.0 CaCl₂, 26.0 NaHCO₃, and 10.0 Glucose. After 15-20 min, the holding chamber was reduced to room temperature and slices were further incubated for 60 min before recording.

3.2.2. Whole-cell recording procedures

Prior to recording, individual slices were transferred to a recording chamber that was maintained at room temperature with oxygenated physiological saline which was recirculated at 2.5-3 ml/min. Individual neurons were identified using DODT contrast optics while whole-cell recordings were obtained using recording pipettes with a tip resistance of 3-6 MΩ. For voltage-clamp recordings the recording pipettes contained (in mM): 117.0 Cs-gluconate, 13.0 CsCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid
(EGTA), 10.0 N-(2-hydroxyethyl) piperazine-N’-(2-ethanesulfonic acid) (HEPES), 2.0 Na₂-ATP, and 0.4 Na-GTP (pH 7.3, 290 mosm). For current-clamp recordings pipettes contained (in mM): 117.0 K-gluconate, 13.0 KCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 EGTA, 10.0 HEPES, 2.0 Na₂-ATP, and 0.4 Na-GTP (pH 7.3, 290 mosm). Pipettes also contained Alexa 594 (50 μM) to allow for imaging. The pipette solutions resulted in a junction potential of approximately 10 mV and was corrected for in all voltage recordings. During recordings the pipette capacitance was neutralized and the access resistance was continually monitored.

Pharmacological agents were prepared and stored as recommended by the manufacturer, and subsequently diluted in physiological saline just prior to use. All pharmacological agents were bath applied at least 10-15 min before subsequent experimental tests. Tetrodotoxin (TTX), 6, 7-dinitroquinoxaline-2,3-dione (DNQX), 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridazinium bromide (SR95531), cis-[Ru(bpy)₂(PMe₃)GluH₂](PF₆)₂, with bpy =2,2′bipyridine and PMe₃= trimethylphosphine (RuBi-Glutamate), and 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) were all purchased from Tocris (Ellisville, MO).

3.2.3. Glutamate uncaging and analysis

Photoactivation of RuBi-glutamate (100 μM) was achieved by focusing a one-photon visible laser (405 nm: 100 mW Coherent Cube diode laser, Santa Clara, CA) coupled into the scan head with a photoactivation module and focused at relay neuron dendrite using a set of galvanometers controlled by TriggerSync software (Prairie Technologies, Middleton, WI). The lower concentration of RuBi-glutamate minimized the antagonistic effects of the caged-compound on GABAergic transmission (Fino et al., 2009). Simultaneous imaging of the recorded neuron was performed by laser excitation (820 nm) via a high NA objective using a
custom two-photon laser scanning microscope (2PLSM, Ultima, Prairie Technologies, Middleton, WI) coupled with a Ti-sapphire laser (MaiTai HP, Spectra Physics, Mountain View, CA).

Once a location was selected, RuBi-Glutamate was released with one of two pulse durations: a long duration (100-200 ms, low-intensity) or a short duration pulse (0.1-3.0 ms, high-intensity). The quantification of IPSC activity was accomplished using two different approaches: calculating the synaptic charge and quantifying IPSC frequency. Synaptic charge was estimated by calculating the total area of the response above a simulated direct response (i.e. inward current). The direct responses were recorded in the presence of the GABA\textsubscript{A} receptor antagonist (SR95531: 10 \(\mu\)M), which blocked all IPSCs. The direct response was simulated by constraining a single exponential fit to the peak inward current for each response and the average time constant calculated from a single exponential fit to the direct response (\(\tau=799\pm226\); \(r^2=0.79\pm0.06\), \(n=10\)). Quantification of IPSC frequency was accomplished using Mini-Analysis software (Synaptosoft, Leonia, NJ). All events were detected automatically by the software and verified post hoc by visual analysis. The threshold for IPSC detection was established from the baseline noise level recorded in the presence of SR95531 (10 \(\mu\)M). For quantification, the average IPSC frequency was calculated from 0.5-sec time windows. The change in IPSC frequency in response to stimulation was determined by comparing the post-stimulus frequency in response to glutamate release (long pulse: 1.0 sec; short pulse: 0.5 sec) to the average pre-stimulus frequency (long pulse: 10 sec; short pulse: 1.0 sec). The average change in IPSC frequency was than calculated for each location across consecutive stimulations (long pulse: 3 stimulations, 80-240 sec interstimulus interval; short pulse: 20 stimulations, 10-20 sec interstimulus interval). It is important to note that in some cells that responded to the glutamate
application with a highly synchronous outward current presumably consisting of temporal summation of multiple IPSCs, our frequency analyses has likely underestimated the absolute magnitude of the response. Nonetheless, our quantified data using both analyses approaches in the same neuronal population yielded comparable results (e.g. Fig 2D). For the mapping study, four locations (25-30 µm apart) were tested for each dendrite with 2-5 dendrites sampled per neuron.

The extent of glutamate diffusion following photo-release was determined by varying the lateral distance between the dendrite and the center of the light beam (Pettit et al., 1997). The relationship between lateral position and the magnitude of the glutamate response was described by a single Gaussian function, and the half-width was used to determine radial spread. Experiments were performed while in TTX (1 µM) on individual dendrites of thalamic reticular and layer IV neocortical neurons because the dendritic pattern of relay neurons made it difficult to isolate individual branches. Unless otherwise indicated, all population data are expressed as mean ± standard deviation and significance defined as p<0.05 using a paired t-test. We express the total n as the number of dendrites (D) sampled from all neurons (N).
3.3.0. Results

3.3.1. iGluR-dependent activation of F2 terminals

To monitor dendritic output of interneurons located in the rat dorsal lateral geniculate nucleus (dLGN), we recorded inhibitory postsynaptic currents (IPSCs) from thalamocortical relay neurons using an *in vitro* thalamic slice preparation (Fig. 13b) (Cox and Sherman, 2000; Govindaiah and Cox, 2004). Relay neurons were distinguished from interneurons by both physiological and morphological criteria (Pape and McCormick, 1995; Williams et al., 1996). IPSC activity was recorded in voltage-clamp mode using a cesium-based pipette solution and command voltage of 0 mV to optimize these currents. To stimulate GABA release from dendritic F2 terminals caged-glutamate was photo-released near a dendrite of a recorded relay neuron filled with a fluorescent indicator (Alexa 594: 50 µM) and visualized using a two-photon laser-scanning microscope (820 nm, Fig. 13b, c). A low concentration of RuBi-glutamate (100 µM) was used to reduce the antagonism of inhibitory transmission caused by caged compounds (Fino et al., 2009). At this concentration RuBi-glutamate produced a 23±11% and 11±6% reduction in the miniature IPSC frequency and amplitude, respectively (n=6 neurons (N)). Considering glutamate release could generate Na⁺-dependent action potentials in interneurons, which could lead to increased axonal output (F1 terminal), all experiments were performed in tetrodotoxin (TTX: 1 µM) to block action potentials.

To increase the probability of stimulating a presynaptic terminal, we initially used a long duration laser pulse (100-200 ms, 405 nm, and low intensity) to release glutamate over a larger area. To determine the extent of glutamate diffusion (i.e. stimulation size) we calculated the half-width of a Gaussian function fit to glutamate responses obtained when moving the laser laterally
across the dendrite of a recorded neuron (see methods). Using this method we estimate that the radial spread of glutamate was 11.0±3.5 µm (200 ms laser pulse; n=5N). To ensure that the laser pulse repeatedly and reliably produce a consistent response at a single location, regardless of tissue depth or opacity, we measured the direct response onto a relay neuron across three consecutive stimulations (80 sec interstimulus interval). When held at -65 mV (in TTX: 1 µM) we observed no significant difference between the amplitude of the first (4.76±1.91 mV) and third response (4.71±2.01 mV, n=20 dendrites (D)/5 neurons (N); 200 ms laser pulse; p=0.75). This suggests that the stimulation parameters produced a stable glutamate response and had no toxic side effects.

As expected, photo-release of glutamate produced a brief inward current in the relay neuron, which likely arises from direct glutamate stimulation of the postsynaptic relay neuron (Fig. 13d, negative response). However, we often observed that the initial inward current was abruptly shortened by a strong outward current and brief increase in IPSC activity (Fig. 13d, positive response). Positive responses were defined as an increase in IPSC activity that exceeded two standard deviations above baseline IPSC frequency and were qualitatively similar to the TTX-insensitive, F2-dependent responses previously described (Cox and Sherman, 2000; Cox et al., 1998). Subsequent application of the GABA\textsubscript{A} receptor antagonist, SR95531 (10-20 µM), completely blocked the evoked IPSC activity (n=16D/5N), which in turn unmasked the inward current (Fig. 13d). The majority (53%) of dLGN neurons recorded produced only negative responses, while the remaining (47%) generated both positive and negative responses (n=51N). This is consistent with previous anatomical studies indicating not all dLGN relay neurons are innervated by F2 terminals (presynaptic dendrites; Sherman, 2004).
To confirm that the change in inhibitory activity resulted from a glutamate-mediated release of GABA, we tested the calcium dependence of the positive response. Considering transmitter release from presynaptic dendrites is a calcium dependent process (Acuna-Goycolea et al., 2008; Castro and Urban, 2009; Cox et al., 1998; Murphy et al., 2005), lowering the extracellular calcium concentration should attenuate the evoked IPSC activity. Using a low-calcium (0.2 mM)/high-magnesium (6.0 mM) containing extracellular solution, the glutamate-mediated increase in IPSC activity was significantly attenuated (n=10D/5N; Charge: p<0.001; Frequency: p<0.01; Fig. 14a, d). The suppression of inhibitory activity reversed following a 10-15 minute wash in control extracellular solution.

Since previous studies have demonstrated that type 5 mGluRs (mGluR5) regulate dendrodendritic output at the F2 terminal (Godwin et al., 1996; Govindaiah and Cox, 2006), we proceeded to test if these receptors were involved with the glutamate-mediated increase in IPSC activity. In the presence of the selective mGluR5 antagonist MPEP (30-50 µM), the glutamate-evoked increase in IPSC activity was unchanged (n=6D/4N; Charge: p=0.12; Frequency: p=0.33; Fig. 14b, d). Although previous studies have provided indirect evidence suggesting iGluRs control dendrodendritic output in the thalamus (Acuna-Goycolea et al., 2008; Blitz and Regehr, 2005; Cox and Sherman, 2000), a direct test of whether this occurs local to F2 terminals and independent of axonal output has been lacking. In the presence of the selective AMPA receptor antagonist DNQX (20–40 µM), the glutamate-evoked increase in IPSC activity was completely blocked (n=12D/5N; Charge: p<0.0001; Frequency: p<0.001; Fig. 14c, d). These findings suggest iGluRs are present near or at F2 terminals and are sufficient to initiate a presumed local GABA release independent of mGluR5 being present or activated.
Interestingly, as shown in figure 3, we did occasionally observe a lasting increase in IPSC activity (>5 sec, 9 of 290D: 3%). When we applied the selective mGluR5 antagonist MPEP (30-50 µM) the duration of IPSC activity was reduced in 4 of 6 locations examined (54.3±31.8%, n=4N); however, the initial transient increase persisted. Subsequent addition of the AMPA receptor antagonist DNQX (20 µM) completely attenuated the initial response. This observed prolonged IPSC activity is qualitatively similar to the mGluR5 mediated F2 output we have previously described with bath application of selective agonists and tetanic optic tract stimulation (Govindaiah and Cox, 2004; Govindaiah and Cox, 2006). These data suggest that some F2 terminals are co-regulated by both iGluRs and mGluRs.

3.3.2. Glutamate uncaging results in local release from thalamic interneurons

Critical to our interpretation is that glutamate uncaging results in a local release of GABA from interneuron dendrites. However, global release of GABA from thalamic dendrites has been shown to occur from both the active propagation of single action potentials as well as plateau-like calcium spikes across the dendritic tree (Acuna-Goycolea et al., 2008). So we proceeded to test if our glutamate stimulation caused a local or global excitation of interneurons (i.e., sub- or supra-threshold excitation). Using the same stimulation that increased IPSC activity in relay neurons, we photo-released glutamate along the dendrites of thalamic interneurons while recording the membrane response at the soma (Fig. 16a). At an initial membrane potential of -65 mV, proximal stimulation (0-44 µm from soma) always produced a larger depolarization than distal stimulation (150-263 µm from soma) (Proximal: 12.1±6.4 mV, n=8D/4N; Distal: 3.1±2.3 mV, n=9D/4N; p<0.0001, Student t-test; Fig. 16b). Hyperpolarizing the membrane potential to -
85 mV did not alter the amplitude of either the proximal (p=0.12) or distal response (p=0.80). Given the small amplitude of the responses these data suggest that our stimulation was not sufficient to evoke the large plateau-like calcium spike underlying global dendritic release in mouse interneurons (Acuna-Goycolea et al., 2008). There was also a low probability of action potential discharge with distal stimulation (1 of 9 D: 11%) when compared to proximal stimulation (5 of 8 D: 63%). We never observed action potential discharge from a hyperpolarized membrane potential (Fig. 16c). Since F2 terminals are typically found on distal dendrites (Sherman, 2004), these data strongly suggest that our stimulation was sub-threshold and that GABA release resulted from local actions and not global excitation of thalamic interneurons.

3.3.3 Glutamate-evoked changes in IPSC activity are mediated by F2 and not F1 terminals

It is also possible that glutamate could increase IPSC activity by direct excitation of axonal terminals (F1 terminals) as well. To eliminate this possibility, we performed similar experiments on relay neurons of the rat ventrobasal complex (VB). Unlike the rat dLGN, which contains both F1 and F2 terminals, the rat VB lacks local interneurons and receives F1 input predominantly from thalamic reticular neurons (Arcelli et al., 1997; Ohara and Lieberman, 1993; Ottersen and Storm-Mathisen, 1984). Therefore, a TTX-insensitive increase in IPSC activity recorded in rat VB relay neurons would indicate a direct action on F1 terminals. To compare relay populations we systematically stimulated various dendritic locations in a population of dLGN neurons that displayed positive responses (n=24N) and VB neurons (n=4N; Fig. 17a, b). As expected, the change in IPSC activity was markedly different between relay neurons (p<0.0001, Kolmogorov-Smirnov test), as robust changes were frequently observed in dLGN
(128 of 290D: 44%) and never observed in VB neurons (0 of 64D: 0%; Fig. 15c). Moreover, the change in IPSC activity had a bimodal distribution in dLGN neurons with peaks centered at 13.9 and 133.3%, but only a single peak in VB neurons (-5.4%, Fig. 17d). Based on these findings we are confident that focal glutamate stimulation results in the local and selective activation of dendrodendritic F2 synapses. This local activation led to a significant 175% increase in IPSC activity above the control baseline frequency in the dLGN relay neurons (Control: 5.0±2.0 Hz; Glutamate: 12.6±3.8 Hz, n=128D/24N; p<0.0001).

To determine if iGluR-mediated F2 output could impact the integration of coupled excitatory input (presumed retinogeniculate via the triad), we evoked a transient membrane depolarization in both dLGN and VB neurons before and after application of the GABA_A receptor antagonist SR95531 (10-20 µM; Fig. 17e). Using the same stimulation that increased IPSC activity, the transient membrane depolarization was evoked by stimulating a relay neuron dendrite (25-40 µm from soma) and recorded while holding the soma at -65 mV in current-clamp. At this membrane potential, we avoid activating dendritic T-type calcium channels, which would result in a calcium dependent low-threshold spike (Crandall et al., 2010; Crunelli et al., 1989; Errington et al., 2010; Huguenard and Prince, 1992). Overall, the change in area and amplitude of the evoked depolarization after SR95531 application was much greater for dLGN neurons than VB neurons (dLGN: 74D/19N; VB: 28D/7N; area: p=0.03, amplitude, p=0.09 with a Kolmogorov-Smirnov test; Fig. 17f). These results suggest that an iGluR-mediated activation of a F2 terminal, presumably via a coupled retinogeniculate terminal, would lead to a direct feed-forward inhibition of the local excitatory postsynaptic potential generated in the dLGN neuron.
3.3.4. Organization of dendrodendritic connections between relay neurons and interneuron

Next we wanted to map the synaptic connectivity between the presynaptic dendrites of thalamic interneurons and their relay neuron targets with single-dendrite resolution. To do this we classified the 290 locations tested (n=24N) into 1 of 4 spatially distinct locations based on measured distance from soma (Fig. 18a). The probability of glutamate stimulation resulting in a change in IPSC activity decreased with increased distance from soma (Proximal vs. Distal; p<0.0001 with a Kolmogorov-Smirnov test; Fig. 18b). Overall, positive F2 responses were more frequent at proximal locations (15-25 µm; 52 of 69D: 75.4%) than at distal locations (80-120 µm; 12 of 69D: 17.4%). This physiological data correlates well with previous anatomical studies suggesting retinogeniculate terminals, and thus F2 terminals, are preferentially localized to the proximal dendrites of relay neurons (Sherman, 2004; Wilson et al., 1984).

A closer inspection of the distribution of F2 terminals (i.e., positive responses) suggested they could also be clustered along dendrites of individual relay neurons (Fig. 19a). In order to determine if clustering occurred, we compared the average change in IPSC frequency for each dendrite (i.e., 4 locations) and displayed the results using a polar plot (Fig. 19a-c). Comparisons were made only in neurons which had 3-6 dendrites examined (n=15N). In total, 7 of 15 (47%) dLGN neurons had significant differences in IPSC activity between dendrites (p<0.035 or less, one-way ANOVA), while the remaining neurons had a more distributed distribution (Fig. 19d). These findings imply that F2 terminals can cluster along individual dendrites in a subpopulation of dLGN neurons.
3.3.5. Glutamate uncaging reveals F2 terminals are capable of local input-output computations

Our previous long duration and low-intensity stimulation allowed us to systematically stimulate different parts of the dendritic tree in order to map inhibitory dendrodendritic connections between thalamic interneurons and relay neurons. However, since this method of glutamate application resulted in a stimulated area roughly 20 µm in diameter, it likely activated numerous F2 terminals as well as non-synaptic receptors (Callaway and Yuste, 2002). In order to reduce the stimulated area and to evoke GABA release from fewer F2 terminals while limiting non-synaptic receptor activation, we used a short duration, high-intensity laser pulse (0.1-3.0 ms). We estimated that the radial spread of glutamate using this stimulation was 3.6±0.6 µm (n=4N). To identify a dendritic region innervated by F2 terminals we first used the long duration pulse (Fig. 20a). We next searched that region using the shorter stimulation to locate a branch innervated by a F2 terminal(s) (Fig. 20b, c; Location 5 and 6). Similar to our previous results, the evoked IPSC activity was sensitive to the GABA<sub>A</sub> receptor antagonist SR95531 (10 µM: n=4N; Fig. 20c red traces). Responses were also reliably obtained across consecutive trials (Fig. 20d). To determine the mechanism behind the change in IPSC activity we first tested the effect of different iGluR antagonists. Similar to our previous results (Fig. 16), application of the AMPA receptor antagonist DNQX (20 µM) completely blocked the evoked IPSC activity (n=5N; p<0.01; Fig 8e, h). Next we examined the contribution of NMDA receptors using the receptor antagonist CPP. Bath application of CPP (10 µM) significantly reduced the IPSC activity by 63.6±28.3% (n=6N; p<0.01) and blocked nearly all the inward current (Fig. 20f, h). Finally, we wanted to test the contribution of the L-type voltage-gated calcium channel because previous reports have indicated that this channel plays an important role in GABA release from
interneuron dendrites (Acuna-Goycolea et al., 2008; Errington et al., 2011). Application of the selective L-type channel blocker, nifedipine (10 μM), reduced the evoked IPSC activity by 62.7±29.7% (n=9N; p<0.01) (Fig. 20g, h). This data strongly suggests that individual F2 terminals have the capacity to produce output through the local AMPA/NMDA receptor mediated recruitment of L-type calcium channels, a mechanism similar to that underlying global dendritic release, but one that does not propagate throughout the dendritic arborization (Acuna-Goycolea et al., 2008).
3.4.0 Discussion

In summary, we used glutamate uncaging to examine the nature of local GABA release from the presynaptic dendrites (F2 terminals) of thalamic interneurons. Our results show that output from F2 terminals can be regulated by local iGluRs and that output when coupled with a postsynaptic excitation (presumed retinogeniculate via the triad) can attenuate the magnitude of the excitatory response generated in a thalamic relay neuron. Local dendritic output is strongly dependent on the activation of AMPA receptors and appears partially dependent on NMDA receptors and voltage-gated L-type calcium channels. Moreover, we demonstrate that iGluR mediated release was local in nature, as dendritic stimulation of thalamic interneurons had a low probability of action potential discharge and did not generate a plateau-like calcium spike (Acuna-Goycolea et al., 2008). Our data also provides insight into the functional organization of dendrodendritic synapses in the visual thalamus. We find that presynaptic dendrites are preferentially localized to proximal dendrites of relay neurons. Presynaptic dendrites were also frequently found clustered along dendrites of a single postsynaptic relay neuron. Together, these results imply that thalamic interneurons behave as multiplexors, with hundreds of presynaptic dendritic terminals that operate independent of not only the soma but other terminals as well. Since iGluR mediated dendrodendritic inhibition was not present in all dLGN neurons examined, we believe our results are consistent with in vivo physiology and neuroanatomy described in cat dLGN, where F2 terminals are commonly associated with X but rare for Y thalamic relay neurons (Friedlander et al., 1981; Wilson et al., 1984). Although the distinctions between classes in the rat are not as well established as those in cats and non-human primates, relay neurons in the do display some morphological correlates (Lam et al., 2005). Thus, our findings regarding
local iGluR activation of F2 terminals may provide insight regarding the role of inhibition in the X-visual pathway.

3.4.1. Local dendrodendritic output is controlled by multiple glutamate receptor subtypes

Our finding that F2 terminals are controlled by iGluRs is in agreement with previous observations (Acuna-Goycolea et al., 2008; Blitz and Regehr, 2005; Cox and Sherman, 2000) but differs in that our data suggests that local input is sufficient to produce output. This coupled with previous work examining the function of mGluR5 at F2 terminals (Godwin et al., 1996; Govindaiah and Cox, 2004; Govindaiah and Cox, 2006), suggests that local dendritic release from thalamic interneurons is controlled by two distinct classes of glutamate receptors with different properties (Anwyl, 1999). Activation of iGluRs would occur quickly after afferent input, producing a relatively short-duration output that would influence local excitation in the postsynaptic relay neuron. If iGluRs are found on F2 terminals participating in the synaptic triad (Fig. 13a), it suggests that fast output would limit the effectiveness of the coupled retinogeniculate synapse by providing a direct, short latency source of disynaptic inhibition. With such a tight spatial relationship between inhibitory and excitatory inputs, local dendrodendritic inhibition would likely function to regulate the temporal window for a single retinogeniculate input to integrate with other excitatory inputs (Cruikshank et al., 2007; Pouille and Scanziani, 2001). This could ultimately influence spike output from thalamic relay neurons (Rathbun et al., 2010; Sincich et al., 2007; Usrey et al., 1998). In contrast, activation of mGluRs would occur only during states of high afferent activity, resulting in prolonged inhibitory output and long-lasting influence over the responsiveness of the postsynaptic relay neuron. Such output
occurring within the synaptic triad has been suggested to provide a local gain control for the relay of retinogeniculate information from thalamus to the neocortex (Govindaiah and Cox, 2004; Sherman, 2004).

Can the same F2 terminal be controlled by both iGluRs and mGluRs? Our present study suggests that some F2 terminals are co-regulated by iGluRs and mGluRs, but the evidence for this is largely anecdotal (Fig. 15). In past studies, mGluR dependent release has been isolated using tetanic stimulation of the optic tract or bath application of mGluR agonists in the presence of iGluR antagonists to attenuate potential suprathreshold activation (action potential) of the interneurons (Govindaiah and Cox, 2004; Govindaiah and Cox, 2006). We speculate that the relatively low occurrence of mGluR activation observed in this study is likely a result of the uncaging parameters used, which were likely inadequate to activate mGluRs. Although increasing glutamate concentrations or laser intensity/duration may facilitate mGluR activation, such an approach will also lead to increased GABA antagonism via the caged compound and phototoxicity, respectively (Fino et al., 2009; Svoboda and Yasuda, 2006). Therefore, future studies using different experimental designs will be needed to determine the degree of glutamate receptor co-expression at F2 terminals.

3.4.2. Output flexibility of thalamic interneurons

Most dendrites contribute to neural output by collecting, integrating, and communicating afferent information to the soma/axon, where action potentials occur and communication continues to downstream neurons (Spruston et al., 2008). With presynaptic dendrites the afferent synaptic input is now in close proximity to the synaptic release site, making it possible to couple
local input with proximal output (Margrie and Urban, 2008). This is exemplified by the unique arrangement of the synaptic triad. Here the inhibitory F2 terminal is presynaptic to a relay neuron dendrite and postsynaptic to an excitatory terminal that targets the same relay neuron dendrite (Fig. 13a). The tight coupling within this microcircuit appears to provide the ideal machinery for very localized and independent information processing. The ability perform such actions could allow thalamic interneurons to operate as multiplexors (Bloomfield and Sherman, 1989; Cox et al., 1998), single neurons containing hundreds of independently operating input-output devices. Such a feature is qualitatively similar to that described for amacrine cells, where neurotransmitter release from dendrites is believed to be isolated and reflects the local computation of visual information (Euler et al., 2002; Grimes et al., 2010). If we combine our results demonstrating F2 terminals are capable of local output with the previous finding that interneuron dendrites support active dendritic conductances that initiate neurotransmitter release (Acuna-Goycolea et al., 2008), it suggests that these neurons are capable of generating both local and global output. Such output flexibility could greatly increase the computational power of an individual thalamic interneuron.

3.4.3. The organization of inhibitory dendrodendritic synapses on thalamic relay neurons

The spatial distribution of inhibitory contacts on a target neuron can significantly impact how inhibition influences information processing in the postsynaptic neuron (Markram et al., 2004). Here we provide physiological evidence to suggest inhibitory dendrodendritic synapses are preferentially localized to the proximal dendrites of thalamic relay neurons. This is consistent with anatomical studies indicating primary afferents (i.e., retinogeniculate terminals) and triadic
arrangements are located proximal to the cell body of relay neurons (Famiglietti and Peters, 1972; Guillery, 1969; Montero, 1986; Ralston, 1971). A strong innervation of the proximal region could allow presynaptic dendrites to exert powerful control over the generation and timing of action potentials, a property similar to that of other peri-somatic targeting interneurons in the brain (Markram et al., 2004; Miles et al., 1996; Mittmann et al., 2005).

We also found evidence to suggest dendrodendritic synapses cluster along dendrites in a subpopulation of relay neurons. This observation is intriguing considering retinogeniculate axons can differ greatly with respect to their innervation patterns, strength of connections, and the degree to which their terminals participate in triads (Cleland et al., 1971a, b; Hamos et al., 1987; Usrey et al., 1999). By clustering along a branch, F2 terminals could preferentially influence how excitatory inputs are integrated within the specific branch (Spruston et al., 2008). Another possible function of clustered F2 terminals could be to limit the generation or propagation of dendritic spikes along a given branch (Gasparini and Magee, 2006; Larkum and Nevian, 2008; Losonczy et al., 2008). In the case of thalamic relay neurons, clustered dendrodendritic synapses could regulate calcium signals produced by backpropagating sodium or low-threshold calcium spikes along a specific branch (Crandall et al., 2010; Errington et al., 2010).
3.5.0 Figures

**Figure 13:** Glutamate uncaging results in a TTX-insensitive change in inhibitory activity in dLGN relay neurons. **A,** The basic circuitry of a triad located within a glomerulus. A retinogeniculate (RG) terminal forms an excitatory synapse onto both a thalamocortical (TC) relay neuron and interneuron dendrite (INT: F2 terminal). In turn, the interneuron dendrite (F2 terminal) forms an inhibitory synapse onto the same TC dendrite. **B,** Schematic representing the basic experimental design used to isolate and monitor dendrodendritic activity (F2 terminal output). The green circle indicates an F2 terminal participating in a triad, as shown in **A.** Output from F2 terminals were isolated from axonal (F1 terminal) activity using TTX. F2 output was stimulated by photo-releasing glutamate with a single photon laser (405 nm) along the dendrites of a TC relay neuron and monitored using a cesium (Cs+) based recording pipette and a command voltage of 0 mV. **C,** A 2PLSM image of dLGN relay neuron loaded with Alexa 594 (50 µM). The blue dot indicates the location where a single-photon laser was focused to release RuBi-glutamate (100 µM). The blue shadow illustrates the estimated radial spread of glutamate (see methods). **D,** In TTX (1 µM, black line) a single laser pulse produced one of two responses. The negative response was identified by a brief direct response (i.e., inward current) with little change in IPSC activity. The positive response (obtained from the location shown in **C**) was identified by a robust increase in IPSC activity. Subsequent addition of SR95531 (10-20 µM: red line) attenuated the evoked IPSC activity and unmasked the direct response (i.e., inward current).
Figure 14: iGluRs regulate local dendrodendritic output. A, Left, In TTX (1 µM), glutamate release increased IPSC activity in a dLGN relay neuron. Right, in a low-Ca\(^{2+}\) (0.2 mM)/high-Mg\(^{2+}\) (6.0 mM) extracellular solution, the evoked IPSC activity was significantly attenuated. Responses recovered after a 10-15 min wash (data not shown). B, Left, in a different neuron (while in TTX: 1 µM), glutamate release increased IPSC activity. Right, addition of the mGluR\(_5\) antagonist MPEP (30-50 µM) did not change the evoked IPSC activity. C, Left, in a different neuron (while in TTX: 1 µM), glutamate release increased IPSC activity. Right, addition of the AMPA receptor antagonist DNQX (20-40 µM) significantly reduced the evoked activity. D, Population data illustrating the decrease in IPSC charge and frequency by both low-Ca\(^{2+}\) and DNQX.
Figure 15: Dendrodendritic synapses are regulated by both iGluRs and mGluRs. **A, Top**, an example dLGN relay neuron in which focal glutamate release (indicated by the black dot) produced a prolonged change in IPSC activity (in TTX: 1 µM). **Right**, expanded trace of the period represented by the gray line. **Middle**, addition of the mGluR$_2$ antagonist MPEP (50 µM) significantly reduced the duration of IPSC activity, but had minimal effect on the magnitude of the initial response. **Bottom**, Subsequent application of DNQX (20 µM) blocked the initial increase in IPSC activity.
Figure 16: Glutamate uncaging generates local and not global GABA release from thalamic interneurons. **A,** Right, A 2PLSM image of a local thalamic interneuron filled with Alexa 594 (50 µm). Without TTX, glutamate uncaging (white shadows) was performed at various locations (L) while recording the voltage response at the soma. Left, Somatic voltage recordings from each location while holding the soma at a depolarized (-65 mV) and hyperpolarized (-85 mV) membrane potential. The star (*) indicates a truncated action potential while the block dot represents when the laser pulse was delivered. A plateau-like calcium spike (Acuna-Goycolea et al., 2008) was never generated with focal glutamate stimulation. **B,** Population data indicating that the response amplitude was independent of holding potential and was dependent on location (Proximal: 0-44 µm; Distal: 150-263 µm). **C,** Population data indicates that the probability of spiking was highest when stimulating proximal locations at a depolarized membrane potential. No spiking was observed when the cell was hyperpolarized.
Figure 17: Glutamate uncaging produces a TTX-insensitive change in IPSC activity in rat dLGN but not VB neurons. A, Left, 2PLSM image of a dLGN relay neuron. The blue shadows indicate two locations where glutamate was released. Right, In TTX (1 µM), glutamate released at location 1 produced a change in IPSC activity (top: positive response). Glutamate released at location 2 did not alter IPSC activity (bottom: negative response). B, Left, 2PLSM image of a VB relay neuron. Right, In TTX (1 µM), glutamate released at locations 1 and 2 produced no change in IPSC activity (negative response). C, Cumulative probability graph showing that the change in IPSC frequency was significantly different for VB (red) compared to dLGN neurons (green). D, Shown is a relative frequency histogram of the change in IPSC frequency for both dLGN (green) and VB neurons (red). The dLGN population was best fit by a double Gaussian curve while a single curve best fit the VB population (dashed lines). Bin size = 12%. E, Current-clamp recordings obtained from dLGN and VB neurons after glutamate stimulation in the presence of TTX (1 µM: black) (dLGN: amplitude: 8.6±5.1 mV; area: 3092±2721 mV*ms, n=74D/19N; VB: amplitude: 4.2±1.7 mV; area: 1142±695 mV*ms, n=28D/7N). Subsequent application of SR995531 (10-20 µM: gray) frequently enhanced the transient membrane depolarization evoked in dLGN neurons. Responses recovered after a 10-15 min wash (data not shown). F, Cumulative probability graphs showing SR95531 increased the area and amplitude of the evoked depolarization in dLGN neurons more than in VB neurons.
Figure 18: Inhibitory dendrodendritic synapses are preferentially localized to the proximal dendrites of relay neurons. A, A 2PLSM image of a dLGN relay neuron filled with Alexa 594 (50 µM). Glutamate was released at four spatially distinct locations along individual primary dendrites. As measured from the soma: (1) Proximal: 15-25 µm, (2) Intermediate I: 30-45 µm, (3) Intermediate II: 50-75 µm, (4) Distal: 80-120 µm. Shown are the corresponding responses for each of the locations tested. B, A cumulative probability graph for each dendritic location examined. The probability of observing a change in IPSC activity decreased from proximal to distal locations.
**Figure 19:** Distribution of dendrodendritic synapses across individual dLGN neurons.  

**A,** A 2PLSM image of a dLGN relay neuron filled with Alexa 594 (50 µM). The image illustrates the spatial distribution and response magnitude for each of the distinct locations tested from this neuron. Note that glutamate stimulation along one of the dendrites consistently evoked a robust change in IPSC activity independent of location. Stimulation along other dendrites produced either weak changes in IPSC activity or no response (NR) at all. The small inset shows both location and orientation of the cell within the dLGN (coronal plane).  

**B,** Polar plot of the 4 branches tested as a function of branch orientation (dorsal (D), lateral (L), ventral (V), and medial (M)). Plotted are the average baseline frequencies (blue) and glutamate evoked frequencies (green) for each dendrite. The average for each dendrite was calculated by combining the responses obtained from each of the 4 locations (3 stimulations per location). **C,** Shown is a polar plot of the average change in IPSC frequency for each dendrite shown in (B). A one-way ANOVA was used to compare dendrites across a single neuron.  

**D,** Top, example of 2 dLGN neurons with clustered F2 innervation patterns (Cell 1 and 2). Note that only 3 of the 5 dendrites examined in Cell 1 were innervated by F2 terminals. Below, example of 2 dLGN neurons with distributed F2 innervation patterns (Cell 3 and 4).
Figure 20: Focal glutamate uncaging reveals the mechanism behind local dendrodendritic output. 

A, A 2PLSM image of a dLGN relay neuron filled with Alexa 594 (50 µM). In order to use a more focal stimulation we first used a long-duration laser pulse (blue shadow: 100 ms, low-intensity) to locate a region innervated by F2 terminals. Below, in TTX (1 µM) a long stimulation produced a robust change in IPCS activity. 

B, A high resolution image indicating where focal glutamate uncaging (blue spots) was performed within the selected region shown in A. 

C, Ten random responses for the six locations are shown. Application of SR95531 (10 µM: red traces) attenuated the evoked IPSC activity at location 6. 

D, Population data (mean ± SEM) indicates that the increase in IPSC activity can be reliably obtained in consecutive trials (0.05 Hz interval). 

E, Left, a 2PLSM image of the region where focal uncaging was performed (Pulse: 1.0 ms). Middle, ten random responses obtained in TTX (1 µM). Right, subsequent addition of DNQX (20 µM) completely blocked the evoked IPSC activity. 

F, Left, a 2PLSM image of the region where focal uncaging was performed (Pulse: 0.1 ms). Middle, ten random responses obtained in TTX (1 µM). Right, subsequent addition of CPP (10 µM) significantly reduced the evoked IPSC activity and blocked the inward current. 

G, Left, a 2PLSM image of the region where focal uncaging was performed (Pulse: 2.0 ms). Middle, ten random responses obtained in TTX (1 µM). Right, subsequent addition of Nifedipine (NIF: 10 µM) significantly reduced the evoked IPSC activity. 

H, Population data summarizing the effect each receptor antagonist had on the evoked IPSC frequency. The asterisks indicate statistical significance.
3.6.0 References


Thalamic microcircuits: presynaptic dendrites form two distinct feedforward inhibitory pathways in thalamus

4.0.0. Abstract

In the visual thalamus, retinal afferents activate both local interneurons and excitatory relay neurons, leading to a robust feedforward inhibition that regulates the transmission of sensory information from retina to neocortex. Peculiarly, this feedforward inhibitory pathway is dominated by dendrodendritic synapses. Little is known, however, regarding how different classes of glutamate receptors regulate local GABA release from interneuron dendrites. Here we used focal glutamate uncaging and whole-cell recordings to reveal distinct types of dendrodendritic synapses in the visual thalamus of the rat. These inhibitory dendrodendritic synapses target the same postsynaptic neurons and were exclusively mediated by ionotropic or a combination of ionotropic and metabotropic glutamate receptors. The segregation of synapses, based on glutamate receptor expression, suggests these dendrodendritic synapses serve to differentially alter the incoming sensory signals in an activity-dependent manner. Undoubtedly, these two inhibitory microcircuits will play an important role in processing visual information in thalamus.
4.1.0. Introduction

The thalamus is the entry point for which sensory information must pass prior to entering the neocortex. In the visual thalamus (i.e., dorsal lateral geniculate nucleus: dLGN), there are two classes of neurons excited by retinal afferents: excitatory relay neurons that project to layer IV visual cortex and inhibitory interneurons that project to relay neurons and each other (Sherman and Guillery, 1996). The GABAergic connections made by thalamic interneurons, in turn, form a retinogeniculate driven feedforward inhibitory circuit, which is thought to enhance stimulus selectivity, improve sensory coding, and ensure temporal precision of spiking (Wang et al., 2011).

Peculiarly, this local inhibitory circuit is dominated by dendrodendritic synapses formed between dendrites of interneurons and relay neurons (Famiglietti and Peters, 1972; Guillery, 1969; Hamos et al., 1985; Lieberman, 1973; Montero, 1986; Ohara et al., 1983; Ralston, 1971). These dendritic terminals (i.e., F2 terminals) are often found in triads, a synaptic arrangement in which an interneuron dendrite is presynaptic to a relay neuron dendrite and postsynaptic to an excitatory retinogeniculate terminal that, in turn, targets the same postsynaptic relay dendrite (Sherman, 2004). Since presynaptic F2 terminals are located on distal dendrites of interneurons and are in close proximity to excitatory input, these terminals are thought to integrate local subthreshold synaptic information independently of the axon as well as each other (Bloomfield and Sherman, 1989; Cox et al., 1998; Crandall and Cox, 2012). In theory, this allows thalamic interneurons to operate as multiplexors, containing numerous independently operating input-output devices (i.e. F2 terminals).

Since F2 terminals are postsynaptic to excitatory afferents (Hamos et al., 1985), glutamate must play a major regulatory role in dendrodendritic communication in thalamus.
Currently, there is substantial evidence suggesting GABA release from F2 terminals is regulated by the activation of metabotropic glutamate receptors (mGluRs) (Cox and Sherman, 2000; Cox et al., 1998; Godwin et al., 1996; Govindaiah and Cox, 2004; Govindaiah and Cox, 2006), and ionotropic glutamate receptors (iGluRs: AMPA and NMDA) (Acuna-Goycolea et al., 2008; Blitz and Regehr, 2005; Cox and Sherman, 2000; Crandall and Cox, 2012). However, it is unclear whether different F2 terminals can vary in their complement of receptors. Differences in glutamate receptor expression should ultimately serve to differentially alter the incoming sensory signals in an activity-dependent manner. Addressing this question is a critical step toward understanding how thalamic interneurons process and modulate visual information prior to entering the neocortex.

In this study, we used focal glutamate uncaging to study local synaptic connections between presynaptic dendrites of inhibitory interneurons and relay neurons (Crandall and Cox, 2012). Combining this approach with whole-cell recordings and two-photon imaging, we identified two types of dendrodendritic synapses based on their glutamate receptor regulation. Our data suggest that the efficacy by which the retina communicates with the thalamus, and subsequent transfer to the neocortex, may be strongly regulated by the activity level of retinal ganglion cells and the specific feedforward inhibitory pathway engaged by the retinogeniculate afferents.
4.2.0. Methods and materials

4.2.1. Slice preparation

All experimental procedures were carried out in accordance with the National Institute of Health guidelines for the care and use of laboratory animals and approved by the University of Illinois Animal Care and Use Committee. Tissue sections were prepared from young Sprague-Dawley rats (postnatal age: 13-20 days) of either sex as previously described (Crandall et al., 2010). Briefly, rats were deeply anesthetized with pentobarbital sodium (50 mg/kg) and perfused with cold, oxygenated slicing solution before being decapitated. The brains were quickly removed and immediately placed in cold (4°C) oxygenated (5% CO₂, 95% O₂) slicing solution containing (in mM): 2.5 KCl, 1.25 NaH₂PO₄, 10.0 MgSO₄, 0.5 CaCl₂, 26.0 NaHCO₃, 10.0 glucose, and 234.0 sucrose. Coronal slices (~300 µm) containing dLGN were obtained using a vibrating tissue slicer (Leica, Germany). Once sliced, tissue sections were immediately transferred to a holding chamber containing oxygenated, physiological saline solution and incubated for 15-20 min at 32±1 °C and an additional 60 min at room temperature. The physiological solution was continuously oxygenated (5% CO₂, 95% O₂, pH of 7.4) and contained in mM: 126.0 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 MgCl₂, 2.0 CaCl₂, 26.0 NaHCO₃, and 10.0 Glucose.

4.2.2. Whole-cell recording procedures

For recording, individual tissue sections were transferred to a submersion type recording chamber maintained at 32±1 °C and continuously superfused (2.5-3 ml/min) with recirculating oxygenated physiological saline. Individual neurons were visualized using an Olympus BX-51WI fixed stage microscope equipped with DODT contrast optics and a water-immersion
Electrophysiological data was acquired using a MultiClamp 700A amplifier, filtered at 2-3 kHz and digitized at 10 kHz using a Digidata 1440A digitizer in combination with pClamp10 software (Molecular Devices). For voltage-clamp recordings, patch pipettes had a tip resistance of 3-6 MΩ when filled with a cesium-based internal solution containing (in mM): 117.0 Cs-gluconate, 13.0 CsCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 10.0 N-(2-hydroxyethyl) piperazine-N'- (2-ethanesulfonic acid) (HEPES), 2.0 Na₂-ATP, and 0.4 Na-GTP (pH 7.3, 290 mosm). To better isolate inhibitory postsynaptic currents (IPSCs), all voltage-clamp experiments were performed at a command voltage between 0 and +10 mV after correcting for a liquid junction potential (~10 mV). For current-clamp recordings pipettes contained (in mM): 117.0 K-gluconate, 13.0 KCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 EGTA, 10.0 HEPES, 2.0 Na₂-ATP, and 0.4 Na-GTP (pH 7.3, 290 mosm). Pipettes also contained Alexa 594 (50 μM) to allow for imaging. During recordings the pipette capacitance was neutralized and access resistance was continually monitored.

Pharmacological agents were prepared and stored as recommended by the manufacturer, and subsequently diluted in physiological saline just prior to use. All pharmacological agents were bath applied at least 10 min before subsequent experimental tests. Tetrodotoxin (TTX), 6, 7-dinitroquinoxaline-2,3-dione (DNQX), 3-((R)-2- Carboxypiperazin-4-yl)- propyl-1-phosphonic acid (CPP), and 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) were all purchased from Tocris Bioscience. cis-[Ru(bpy)₂(PMe₃)GluH₂](PF₆)₂, with bpy = 2,2′-bipyridine and PMe₃ = trimethylphosphine (RuBi-Glutamate), 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)- pyridazinium bromide (SR95531), and (S)-(+-)α-Amino-4-carboxy-2-methylbenzeneacetic acid (LY367385) were purchased from Tocris Bioscience and Ascent Scientific.
4.2.3. Two-photon imaging and single-photon glutamate uncaging

Individual relay neurons were filled via recording pipette with 50 µM Alexa 594 (Molecular Probes) and imaged by laser excitation (820 nm) using a two-photon laser scanning system (Prairie Ultima, Prairie Technologies) coupled with a Ti-sapphire laser (MaiTai HP, Spectra Physics). To photo-release glutamate, a single-photon visible laser (405 nm: 100 mW Coherent Cube diode laser) was coupled into the scan head with a photoactivation module and focused at a relay neuron dendrite using a second set of galvanometers controlled by TriggerSync software (Prairie Technologies). A short duration laser pulse (0.1-3.0 ms) was used and the laser intensity was adjusted to just above response threshold. Bath application of RuBi-Glutamate (100 µM) was chosen because of its high quantum efficiency and reduced antagonistic effects on GABAergic transmission compared to other caged-glutamate compounds (Fino et al., 2009). The extent of glutamate diffusion following photo-release was determined by varying the lateral distance between the dendrite and the center of the light beam using basal dendrites of layer V pyramidal neurons. The relationship between lateral position and the magnitude of the glutamate response was described by a single Gaussian function, and the half-width was used to determine radial spread.

4.2.4. Data analysis

Detection and analysis of IPSC activity were performed off-line using Mini-Analysis software (Synaptosoft). All events were detected automatically by the software and verified post hoc by visual analysis. The amplitude threshold (8-12 pA) was adjusted above baseline noise level recorded in the presence of a GABA_A receptor antagonist (SR95531: 10 µM). Calculating the change in IPSC activity in response to DHPG application was accomplished using two
different measures: IPSC frequency and the root mean square (RMS) of the current recording. The change in IPSC frequency in response to DPHP was determined by subtracting the average baseline frequency, over a 2 min period, from the peak DHPG response, over a 5 sec period. The change in RMS, which is a measure of the power of IPSC activity, was calculated using pClamp10 software. Briefly, the RMS was calculated across a single 10 sec sweep and the change in response to DHPG was determined by subtracting the average baseline activity (2 min; 12 sweeps) from the peak DHPG response over a 10 sec period (1 sweep). To classify a given relay neuron as iGluR-sensitive or iGluR-insensitive, 30-50 different dendritic locations were stimulated with different laser powers and durations. In order for a cell to be classified as iGluR-sensitive, at least one location needed to generate a gluIPSC. For morphological analysis we acquired stacked images of the cell (5.12 pixels/μm; 0.25 μm steps in z-axis) and reconstructed it for analysis using NIH ImageJ Software. Analysis of morphology was done as previously described (Friedlander et al., 1981; Krahe et al., 2011). Briefly, to quantify dendritic polarity, five concentric rings (20 μm intervals) were centered at the soma and then divided into four quadrants. Since the rodent LGN is not a layered structure, as in cat and nonhuman primates, we biased the cell’s orientation so that the axis with the most intersections would be in the a-axial plane (Krahe et al., 2011). The ratio of dendritic intersections (b-axis/a-axis or min/max) was used as an index of dendritic orientation (DOi). When counting branch points, only primary branches that appeared intact (i.e. not cut during the sectioning process) were included. Unless otherwise indicated, population data are expressed as mean ± standard deviation and significance defined as p<0.05.
4.3.0. Results

To explore the functional regulation of inhibitory dendrodendritic synapses, we used single-photon glutamate uncaging to evoke local GABA release from dendrites of rat thalamic interneurons (Crandall and Cox, 2012). Since many caged-glutamate compounds antagonize inhibitory responses, we used RuBi-Glutamate since it has the least potency to inhibit GABAergic transmission and has previously been used to study inhibitory synaptic connections (Crandall and Cox, 2012; Fino et al., 2009; Fino and Yuste, 2011; Packer and Yuste, 2011). At the concentration used in this study, RuBi-Glutamate (100 µM) attenuated the frequency and amplitude of miniature inhibitory postsynaptic currents (mIPSCs) by 30.9±11.3% and 16.7±9.0%, respectively (Control TTX: 9.2±2.6 Hz, 22.2±4.2 pA; +RuBi-Glutamate: 6.4±2.1 Hz, 18.3±2.9 pA, n=6). To locate a synaptic connection between an inhibitory F2 terminal and postsynaptic relay neuron, we photo-released RuBi-Glutamate (0.1-3.0 ms; λ=405 nm) near a dendrite of a dLGN relay neuron filled with Alexa 594 (50 µM) and recorded inhibitory activity in the same relay neuron (Fig. 21A). Because previous anatomical and physiological studies have shown F2 terminals preferentially target the proximal dendrites of relay neurons (Crandall and Cox, 2012; Hamos et al., 1987; Wilson et al., 1984), we chose to focus our search of F2 terminals to dendrites located within 10-50 µm of the soma. To eliminate glutamate-generated action potentials in interneurons and thus axonal output (F1 terminals) from these cells, the voltage-gated sodium channel blocker tetrodotoxin (TTX: 1 µM) was present in all experiments.

When systematically photostimulating the dendrites of relay neurons, we identified an inhibitory F2 terminal as a glutamate-evoked increase in IPSC activity (gluIPSC) that was “time-locked” to the direct photostimulation of the postsynaptic relay neuron (Fig. 21B). GluIPSCs were completely blocked by the GABA\textsubscript{A} receptor antagonist SR95531 (10 µM, n=6) or DNQX.
indicating the responses were mediated by both GABA<sub>A</sub> and AMPA receptors, respectfully (Fig. 21B and 1C). As previously reported, responses were also partially sensitive to CPP (10-20 µM), indicating the involvement of NMDA receptors in GABA release (n=4; data not shown) (Acuna-Goycolea et al., 2008; Crandall and Cox, 2012). These results are consistent with a feedforward inhibitory mechanism that depends on fast glutamatergic excitation of a presynaptic interneuron dendrite and are qualitatively similar to the TTX-insensitive responses we have previously shown to be F2 terminal-mediated (Cox and Sherman, 1999, 2000; Crandall and Cox, 2012). GluIPSCs had an average inward-peak to outward-peak latency of 26.9±8.4 ms and peak amplitude of 37.2±17.1 pA (n=12). In order to determine the size of the active area during photostimulation, we performed additional control experiments in which we measured the relationship between the peak amplitude of a glutamate induced response and the lateral position of the light beam, relative to an isolated dendrite (Fig. 21D). From these experiments, we estimated that the size of the stimulated area was 5.8±1.2 µm (n=5). To gain insight into the magnitude of excitation cause by photostimulation, we also examined the direct excitatory response on primary dendrites (10-20 µm from soma) of relay neurons (Fig. 21E). While in TTX (1 µM) at a membrane potential of -65 mV, photostimulation of relay neurons resulted in a mean amplitude of 1.4±0.4 mV (n=6). Thus, photostimulating local GABA release from a presynaptic dendrite was focal, small amplitude, and reliable.

4.3.1. iGluRs and mGluRs mediate dendrodendritic inhibitory activity in the same relay neuron

Since previous studies have demonstrated dendritic release is regulated by both iGluRs and mGluRs (Acuna-Goycolea et al., 2008; Cox et al., 1998; Crandall and Cox, 2012;
Govindaiah and Cox, 2006), we asked if both classes of glutamate receptors regulated dendritic output onto the same or distinct populations of relay neurons. To address this question, we combined the one-photon photostimulation method with a previously established agonist application method (Cox and Sherman, 2000; Cox et al., 1998), in order to selectively activate F2 terminals regulated by different classes of glutamate receptors. Since glutamate-mediated changes in inhibitory activity are not observed in all relay neurons (Cox et al., 1998; Crandall and Cox, 2012), we initially identified relay neurons as iGluR-sensitive or iGluR-insensitive based on the presence of photostimulated glutamatic glutamate receptor-mediated changes in inhibitory activity. Subsequently, we briefly bath-applied the selective Group I mGluR agonist DHPG (25 µM; 15-25 sec) to test whether the same cell received input from F2 terminals regulated by mGluRs. Previous work has demonstrated that many F2 terminals contain mGluR5 and that DHPG application will activate these terminals, leading to a robust TTX-insensitive increase in IPSC activity (Godwin et al., 1996; Govindaiah and Cox, 2006).

For relay neurons identified as iGluR-sensitive, DHPG application consistently produced a robust increase in IPSC activity as demonstrated by a significant decrease in the inter-IPSC interval (p<0.001, Kolmogorov-Smirnov test; Fig. 22A, B, E). In iGluR-insensitive relay neurons, DHPG application produced a weaker, but statistically significant reduction in the inter-IPSC interval (p<0.001, Kolmogorov-Smirnov test; Fig. 22C, D, E). To test if the magnitude of change was different between identified relay neurons, we compared the peak change in IPSC frequency after DHPG application. On average, DHPG produced a significantly larger change in IPSC frequency in the iGluR-sensitive population than in the iGluR-insensitive population (iGluR-sensitive: 17.6±4.1Hz, n=7; iGluR-insensitive: 8.2±4.1Hz, n=6, p<0.01, Student t-test; Fig. 22F). Similar results were found when calculating the change in the root mean square
(RMS) of the current recording, which is a measure of the power of IPSC activity (iGluR-sensitive: 10.5±3.9 pA, n=7; iGluR-insensitive: 3.8±1.8 pA, n=6, p<0.01, Student t-test; Fig. 22F). These results indicate that dendritic F2 terminals can produce qualitatively different inhibitory responses in the same postsynaptic relay neuron (i.e. fast-transient and slow-longer-lasting).

4.3.2. Morphological correlates

Given the results of the previous experiment and the use of bath applied mGluR agonist, we sought to determine if relay neurons could be grouped into morphologically distinct classes based on inhibitory input from F2 terminals. This is of interest because, for many mammals such as cats and nonhuman primates, the visual system consists of separate but parallel retinogeniculo-cortical pathways (Nassi and Callaway, 2009; Sherman and Spear, 1982). To approximate the total inhibitory input from F2 terminals onto an individual relay neuron, we measured the peak change in IPSC activity, while in the presence of TTX, after brief bath application of DHPG (25 µM; 15-25 sec). In total, 30 rat relay neurons ranging in age from P13-17 were tested and then reconstructed from serial images of the recorded neuron obtained from the two-photon microscope. Overall, the change in IPSC activity in response to DHPG was normally distributed (p=0.23, Shapiro Wilk test; Range: Min=3.7 Hz, Max=24.8 Hz, n=30). Similar results were obtained when measuring the change in RMS in response to DHPG (p=0.07, Shapiro Wilk test; Range: Min=-0.5 pA, Max=17.1 pA, n=30). There was no correlation between soma size and DHPG sensitivity (R=0.02, p=0.93, n=29, data not shown). Since the population was normally distributed, our investigation was focused only on relay neurons with ‘strong’ and
‘weak’ F2 input, as determined by their DHPG-mediated change in IPSC activity (Strong: ≥20 Hz, n=9; Weak: ≤10 Hz, n=8; Fig. 23A and 3B).

To assess if dendritic structure was related to the magnitude of F2 input, we used a Sholl ring analysis to quantify dendritic arborization (Friedlander et al., 1981; Krahe et al., 2011). For each neuron, five concentric rings at 20 µm intervals were centered on the soma (Fig. 23C). The outermost ring (100 µm) was large enough to encompass all but the tips of the longest dendrites. For both cell types, the number of dendritic intersections increased initially, peaking 40-60 µm from soma, and then declined with increasing distance (Fig. 23D). Overall, there was no significant difference in the overall branching pattern between cell types (Strong vs. Weak: p=0.83, two-way ANOVA). Moreover, the two cell types did not significantly different in total number of dendritic intersections (Strong: 134±15, n=9; Weak: 133±24, n=7; p=0.89, student t-Test).

To assess if dendritic geometry was related to the magnitude of F2 input, the dendritic arbors were divided into four quadrants by two lines passing through the center of the soma at right angles (Fig. 23C). Relay neurons were oriented so that the maximize number of dendritic intersections (quantified by Sholl analyses) occurred along the a-axial plane (Krahe et al., 2011). The ratio of dendritic intersections in the b-plane versus the a-plane (i.e. minimum/maximum) was taken as an index of dendritic orientation (DOI). Therefore, a purely symmetrical neuron would have a DOI of 1.0 and a purely bipolar neurons would be 0.0, (Krahe et al., 2011). Overall, the dendritic geometries of the two populations were not significantly different (Strong: DOI=0.61±0.18, n=9; Weak: DOI=0.64±0.14, n=7; p=0.80, student t-Test; Fig. 23E).

Although we did not find differences in dendritic geometry, relay neurons with strong DHPG-mediated increases in IPSCs did have significantly fewer primary branches (Strong:...
7.6±1.6, n=8; Weak: 9.9±1.8, n=8, p<0.05, student t-Test; Fig. 23F). Considering cell types did not differ in branching pattern or total number of dendritic intersections (Fig. 23D), these results suggested that relay neurons with strong DHPG-mediated increases in IPSCs would have more branch points per branch. To investigate this we proceeded to count the total number of branch points per intact primary branch for the two classes of relay neurons. As shown in Fig. 23G, the median number of branch points per primary branch was greater for neurons that responded robustly to DHPG than those that responded weakly (Strong: median=8 branch points; range=1-26; n=9 neurons and 53 branches; Weak: median =5; range=0-15; n=8 neurons and 48 branches; p<0.01, Mann-Whitney U-test). These results suggest that arbor complexity, as measured by branch points, and not dendritic geometry is a key morphological feature associated with rat relay neurons innervated by inhibitory F2 terminals.

4.3.3. Two distinct types of F2 terminals mediate dendrodendritic inhibition in thalamus

From our earlier studies, we found that not only do iGluR and mGluR mediated output from F2 terminals presynaptic to the same cell, but these dendritic terminals preferentially target a morphologically distinct population of rat relay neurons. We next asked if iGluRs and mGluRs regulated dendrodendritic inhibition through the same or distinct F2 terminals. To address this, we wanted to photostimulate a local iGluR- and mGluR-mediated response from a single F2 terminal. Considering mGluR-mediated responses in dLGN are only evoked following high frequency electrical stimulation of afferent fibers (i.e., tetanus stimulation) (Govindaiah and Cox, 2004; McCormick and von Krosigk, 1992), we first determined if photo-releasing glutamate could repeatedly and reliably evoke a local mGluR response in our slice preparation. This was done by directly stimulating a relay neuron dendrite with a tetanus photostimulation (10 pulses,
1.5 ms duration, 100 Hz; Fig. 24A). To better isolate the response, experiments were performed with SR95531 (10 µM) and TTX (1 µM) in the bath to eliminate any glutamate evoked GABAergic activity or action potentials, respectively. Since previous anatomical and physiological studies have shown that mGluRs on relay neurons are opposite corticothalamic terminals located along intermediate-distal dendrites (Godwin et al., 1996; McCormick and von Krosigk, 1992; Wilson et al., 1984), photostimulation was limited to second order branches (25-60 µm from soma).

At a membrane potential of -65 mV, tetanus photostimulation of a relay neuron dendrite resulted in a fast followed by a slow depolarizing response in 13 of 16 neurons examined (Fast depolarization: peak: 8.6±4.5 mV; latency to peak: 69.9±22.8 ms, n=13; Fig. 24B). These responses were noticeable different in both magnitude and duration than those observed after a single laser photostimulation (Fig. 21E). Subsequent application of the AMPA receptor antagonist DNQX (20-40 µM) and NMDA receptor antagonist CPP (10-20 µM) blocked the fast potential but did not eliminate the slow depolarization (peak: 1.1±0.5 mV; latency to peak: 1.2±0.5 sec; area: 5040±2053 mV*ms, n=8; Fig. 24C). The long-lasting duration of the slow response (Duration: 11.5±2.9 sec; Range: 8-17 sec) is consistent with mGluR activation and is qualitatively similar to responses observed after a high frequency stimulation of corticothalamic fibers (McCormick and von Krosigk, 1992). Subsequent application of the mGluR1 antagonist LY367385 (100 µM) resulted in a significant attenuation of the amplitude and area of the slow depolarization (peak: 0.4±0.2 mV, n=7, p<0.001; area: 228±422 mV*ms, n=7, p<0.0001, paired t-Test; Fig. 24D and E). These results demonstrate that local tetanus photostimulation can be repeatedly and reliably activate local mGluRs in slice.
To stimulate mGluR-mediated output from inhibitory presynaptic F2 terminals, we first located a dendrodendritic synapse by photostimulating with a single laser pulse (iGluR-sensitive response; Fig. 25A). We subsequently stimulated the same location with a tetanus photostimulation (10 pulses, 1.0-3.0 ms, 100 Hz). As illustrated in Fig. 25B and 5C, tetanus photostimulation produced two distinct responses. The first, which we are calling a ‘Type-A F2 Terminal’ (iGluR-positive and mGluR-negative), was characterized by a transient increase in glutamatergic IPSC magnitude with no lasting changes in IPSC activity (Fig. 25B and 5D). The second, which we are calling a ‘Type-B F2 Terminal’ (iGluR-positive and mGluR-positive), was characterized by an increase in glutamatergic IPSC magnitude followed by a robust and prolonged change in IPSC activity that lasted many seconds (Fig. 25C and 5E). Overall, the time course of IPSC activity in response to a single laser pulse was similar for both types of terminals (Fig. 25F). In contrast, the time course of IPSC activity in response to a tetanus photostimulation was strikingly different between terminals (Fig. 25G). Type A terminals responded to tetanus photostimulation with a fast transient-like response that peaked within the first second following stimulation (10.9±6.4 Hz, n=14) and quickly returned to baseline. Type B terminals, on the other hand, responded to tetanus photostimulation with IPSC activity that peaked 1-3 seconds following tetanic stimulation (12.4±3.4 Hz, n=9) and had a duration of 8.9±2.6 sec (range: 5-13 sec, n=9). Surprisingly, we observed Type-B terminal in only 12 of 38 dendrodendritic connections tested (30.8%; from 34 different neurons), suggesting inhibitory dendrodendritic synapses in the thalamus mainly consist of the Type-A F2 terminal (69.2%).

Curiously, the glutamatergic IPSCs evoked from Type-B terminals were not simply an increase in tonic IPSCs, but they routinely appeared in clusters or bursts (Fig. 25C, bottom inset). Clusters appeared as summated miniatures that resulted in a large amplitude compound IPSC. In total, 7
of 12 Type-B terminals had easily identifiable clusters for quantification. Of these terminals, clusters began appearing 0.5-1.0 seconds following the initial response and produced an average of 8.1±4.2 bursts per stimulation, which was significantly more than after a single laser pulse (1.0±0.9 bursts, n=7, p<0.01, paired t-test). IPSC clusters had a mean peak-to-peak interval of 411±212 ms or occurred at a frequency of 3.0±1.5 Hz (n=209 bursts from 7 neurons).

Overall, the longer time course of the output generated by the Type-B terminal is characteristic of mGluR activation (Govindaiah and Cox, 2004; McCormick and von Krosigk, 1992). To test the potential contribution of mGluRs, we used the selective mGluR5 antagonist MPEP because it has been shown to attenuate dendrodendritic activity in thalamus (Govindaiah and Cox, 2006). In MPEP (50 µM), the evoked increase in IPSC activity was strongly attenuated (Fig. 26A). The initial response, which was unaffected by MPEP, was subsequently blocked by DNQX (20 µM, n=6; Fig. 26A, bottom trace). In Fig. 26B, the time course of IPSC activity before and after MPEP application is summarized for 6 of 8 terminals (8 neurons) that maintained stable baseline activity throughout the recording. Overall, the duration (Control: 7.4±2.5 sec; +MPEP: 2.2±1.2 sec, n=6, p<0.01, paired t-test) and peak increase in IPSC activity in response to tetanus stimulation was significantly reduced in MPEP (Control: 12.5±2.7 Hz; +MPEP: 8.6±2.1 Hz, n=6, p<0.01, paired t-test). These results indicate that the prolonged inhibitory output produced by Type-B F2 terminals, in response to tetanus photostimulation, is dependent on the activation of mGluRs. In addition, this also suggests that output from Type-B terminals and not Type-A terminals, may be strongly regulated by the activity level of retinal ganglion cells.
4.4.0. Discussion

In this study, we have used focal glutamate uncaging and whole-cell recordings to investigate the local regulation of feedforward inhibitory microcircuits involving presynaptic dendrites of interneurons in the visual thalamus (i.e., F2 terminals). Here we found two distinct types of F2 terminals based on their complement of glutamate receptors and physiological output. Moreover, we demonstrate that both types of terminals are presynaptic to the same cell, implying these terminals can differentially alter the incoming sensory signals to a given relay neuron. These relay neurons were also distinct morphologically, with dendritic arbors that were more complex than neurons with less F2 terminal innervation.

Type-A F2 terminal are exclusively regulated by iGluRs (i.e., AMPA and NMDA), whereas the Type-B F2 terminals are regulated by both iGluRs and mGluRs (i.e., AMPA, NMDA, and mGluR5). Furthermore, we found that Type-A terminals outnumbered Type-B terminals 7:3, suggesting these terminals are the dominate type of dendrodendritic synapse in the rat visual thalamus. Although there are fewer Type-B F2 terminals, our data suggest they can exert a much more powerful inhibitory control over relay neuron excitability through the activity-dependent activation of presynaptic mGluRs. This activity-dependent difference, suggests that feedforward inhibitory microcircuits involving Type-A and Type-B F2 terminals would serve distinct roles in modulating the efficacy by which the retina communicates with the thalamus, and subsequent transfer to the neocortex. We also speculate that our results might represent the synaptic basis for differential processing of sensory information in thalamus, across species and modalities.
4.4.1. Are Type-A and Type-B F2 terminals directly involved in the retino-geniculate pathway?

Anatomical and physiological studies have demonstrated thalamic interneurons receive glutamatergic input from a number of sources, including retinal, cortical, and axon collaterals of neighboring relay neurons (Acuna-Goycolea et al., 2008; Augustinaite et al., 2011; Bickford et al., 2008; Errington et al., 2011; Govindaiah and Cox, 2004; Hamos et al., 1985; Williams et al., 1996). Since glutamate uncaging serves only to mimic the function of the presynaptic terminal, we cannot be certain of the endogenous source of glutamate for the two types of F2 terminal described in this study. However, we can presume that most if not all the F2 terminals in this study receive driving input from excitatory retinogeniculate terminals. This is because retinal synapses are an abundant source of excitatory input to thalamic interneurons (Erisir et al., 1998; Montero, 1991; Van Horn et al., 2000), with the vast majority targeting F2 terminal rich dendritic appendages rather than the main dendritic trunks (Hamos et al., 1985; Montero, 1986, 1991). Moreover, anatomical studies indicate that most F2 terminals are postsynaptic to excitatory retinogeniculate terminals (Guillery, 1969; Hamos et al., 1985; Montero, 1986; Ohara et al., 1983). Lastly, our data obtained from glutamate uncaging is consistent with previous physiological studies, in which optic tract stimulation evokes either iGluR or mGluR mediated inhibitory output from interneuron dendrites (Acuna-Goycolea et al., 2008; Govindaiah and Cox, 2004). It is therefore likely that both F2 terminals described in this study are predominantly under retinal control.

4.4.2. Correlates between rat and cat dLGN relay neurons

The visual system of many mammals can be divided into several functionally distinct, parallel pathways from retina to cortex, responsible for processing different visual information
In the cat, the Y-, X-, and W- pathways target three physiological and morphological distinct relay cell types in the dLGN (Lennie, 1980; Sherman and Spear, 1982). Although, all three cell type receive input from axons of thalamic interneurons, the dendrites of interneurons preferentially contact X cells (Friedlander et al., 1981; Wilson et al., 1984). Unlike the cat, such a functional organization has not been well established in the rat dLGN (Lam et al., 2005). Limited rat studies have differentiated X-like and Y-like physiology from in vivo recordings (Davidowa et al., 1993; Gabriel et al., 1996; Lennie and Perry, 1981; Reese, 1988), but it remains unknown whether distinct morphologies are associated with distinct physiological properties.

In the cat, X-cells tend to be bipolar in shape with dendrites that are elongated along projection lines, while Y-cells tend to more radially symmetrical (Friedlander et al., 1981). Here we report that dendritic geometry is not a great indicator of F2 innervation onto rat dLGN relay neuron. Nevertheless, relay neurons with strong F2 innervation did have more complex dendritic arborizations than those with weak innervation, as defined by the number of branch points per primary branch. This finding correlates well with our anecdotal evidence that F2 terminals are commonly found, with glutamate uncaging, at or near branch points. The complex arborization of rat relay neurons is also qualitatively similar to those morphological features described in the cat. In that, Y-cell dendrites tend to be large, fairly straight and possess few simple appendages, while X-cell dendrites tend to be think and sinuous, with many grape-like appendages (Friedlander et al., 1981). These grape-like dendritic appendages are frequently found near branch points and often participate in triadic arrangements (Friedlander et al., 1981; Wilson et al., 1984). Although we do not see many dendritic appendages in rat dLGN relay
neurons, the association between branch points and F2 terminals could represent a functional similarity with the cat X-like cells. Nevertheless, any speculation relating rat and cat relay neurons should be cautioned since much of the intermediate population was ignored.

4.4.3. Insight into the functional significance of two F2 terminals

If we presume that both Type-A and Type B F2 terminals are predominately under retinal control, it is unclear if one or both classes of terminals are involved in the synaptic triad. The triad is a unique synaptic arrangement in which an interneuron dendrite (i.e., F2 terminal) is presynaptic to a relay neuron dendrite and postsynaptic to an excitatory retinogeniculate terminal that, in turn, targets the same postsynaptic relay dendrite (Hamos et al., 1985; Sherman, 2004). If both Type-A and Type-B terminals are found to participate in the triadic arrangement, fast glutamate release from a presynaptic retinogeniculate terminal would result in a monosynaptic excitation of both interneuron and relay neuron dendrite, leading to a fast disynaptic inhibition via the F2 terminal. Because the tight spatial relationship of the triad, fast inhibitory output from the F2 terminal would function to regulate the temporal window for a single retinogeniculate excitatory event to integrate with other excitatory inputs (Cruikshank et al., 2007; Pouille and Scanziani, 2001). Such temporal modulation would likely influence spike output (Rathbun et al., 2010; Sincich et al., 2007; Usrey et al., 1998).

Alternatively, during states of high retinogeniculate activity, only Type-B terminals would be engaged in prolonged inhibitory output, given sufficient activation of mGluRs. In theory, the long-lasting output would modulate the balance between excitation and inhibition within the triad and, thus, tonically regulate the gain of an individual retinogeniculate-relay synapse. The increase in inhibitory tone would ultimately reduce the overall strength of the
synapse and therefore decrease the probability of the involved retinogeniculate terminals to contribute to relay neuron spiking.
4.5.0. Figures

Figure 21: Glutamate uncaging evokes iGluR-mediated GABA release from the presynaptic dendrites of thalamic interneurons. A, Image of a rat thalamic relay neuron filled with Alexa594 (50 µM). Inhibitory activity in the cell was monitored using a cesium (Cs+) pipette and a command voltage of 0 mV. Inset, an image showing the dendrite targeted for single-photon glutamate uncaging (blue dot). B, Top, In TTX (1 µM), 10 control responses produced by uncaging glutamate at the location shown in A. Bottom, 10 responses obtained from the same location after adding the GABA<sub>A</sub> receptor antagonist SR95531 (10 µM). C, Top, In TTX (1 µM), 10 control responses from a different relay neuron produced by uncaging glutamate. Bottom, 10 responses obtained from the same location after adding the AMPA receptor antagonist DNQX (20 µM). D, Plot showing the relationship between the axial position of the laser beam and normalized amplitude of the glutamate evoked response. The blue line is a single Gaussian function fit. E, In TTX (1 µM), direct excitatory responses recorded from three different relay neurons. The black line indicates the average of 20 responses (gray traces).
Figure 22: Both iGluRs and mGluRs mediate dendrodendritic activity in the same relay neuron. **Ai,** Image of a thalamic relay neuron. **Aii,** Inset, image of the dendrite targeted for glutamate uncaging (blue dot). **Aiii,** In TTX (1 µM), 5 control responses produced by uncaging at the location shown. The gluIPSC indicates that the cell was innervated by iGluR-sensitive F2 terminals. **B,** Top, subsequent application of the Group I mGluR agonist DHPG (25 µM) to the same neuron produced a robust increase in F2 mediated IPSC activity. Below, expanded traces before (a) and after (b) DHPG application are shown and correspond to the region indicated. **Ci,** Image of a thalamic relay neuron. **Cii,** Inset, image of the dendrite targeted for glutamate uncaging (blue dot). **Ciii,** In TTX (1 µM), 5 control responses produced by uncaging at the location shown. The lack of a gluIPSC indicates that the cell was not innervated by iGluR-sensitive F2 terminals. **D,** Top, subsequent application of DHPG did not produce a robust change in F2 mediated IPSC activity. Below, expanded traces before (a) and after (b) DHPG application are shown and correspond to the region indicated. **E,** Cumulative probability plots showing a significant decrease in the inter-event interval with DHPG application for both cell types. **F,** Summary of the peak change in IPSC frequency and RMS for both populations after DHPG application.
Figure 23: The relationship between morphological feature and F2 innervation for rat thalamic relay neurons. A, Images of thalamic relay neurons with ‘strong’ F2 innervation as determined by their DHPG change in IPSC frequency (Strong: ≥20 Hz). B, Images of thalamic relay neurons with ‘weak’ F2 innervation (Weak: ≤10 Hz). C, Shown is a Sholl ring superimposed on the dendritic tree of a thalamic relay cell. 5 concentric rings with equidistant intervals (20 µm) were centered on the soma and then divided into 4 quadrants (a1, a2 and b1, b2). Cells were then oriented to have the maximum number of intersects in the a-axial plane. D, Plot illustrating the total number of dendritic intersections as measured from the soma. E, Polar plots of the dendritic intersections for neurons that responded robustly and weakly to DHPG application. The thin white lines represent individual cells and were constructed by calculating the percentage of the total intersections within each 11.25° region. Cells were arranged so the quadrant with the most number of intersects was oriented between 0 and 90° (quadrant a1). The red and blue lines represent the average profile. The outermost ring equals 17.2%. An index of dendritic orientation (DOi) for each cell was then determined from the ratio of the minimum to maximum number of intersections in each axial plane (b1+ b2 / a1+a2). F, Summary of the average number of primary (1°) branches for relay neurons with strong sensitivity and weak sensitivity to DHPG. G, Box plots of the total number of branch points for each of the primary branches analyzed for the two populations.
Figure 24: Tetanus photostimulation reliably activates local mGluRs. A, Schematic illustrating how mGluRs were locally activated using a tetanus photostimulation protocol (10 pulses, 1-2 ms, 100 Hz). B, In TTX (1µM) and SR95531 (10 µM), 5 individual responses produced by tetanus photostimulation (gray traces). The onset of the stimulus is shown by the blue dot. Also shown is the average response in black. Under these conditions, tetanus photostimulation resulted in both a fast (f: truncated) and slow (s) excitatory potential. C, Subsequent application of iGluR blockers (DNQX: 20-40 µM; CPP: 10-20 µM) eliminated the fast potential while leaving the slow potential unaffected. D, The slow potential was attenuated with the addition of the Type I mGluR antagonist LY367385 (100 µM). E, Summary of the effects of LY367385 (LY) on the slow excitatory potential isolated with iGluR blockers (Cont).
Figure 25: Local photostimulation reveals distinct types of dendrodendritic synapses in the visual thalamus. A, Left, image of a thalamic relay neuron and 3 locations targeted for glutamate uncaging. Right, inhibitory activity evoked from the 3 different locations after delivering a single and tetanus photostimulation. B, Characterization of a Type-A F2 terminal. Top, shown is baseline IPSC activity in the presence of TTX (1uM). Middle, photostimulation with a single laser pulse produced a fast change in IPSC activity. Bottom, tetanus photostimulation at the same location did not increase the duration of IPSC activity. C, Characterization of a Type-B F2 terminal. Top, shown is baseline IPSC activity in the presence of TTX. Middle, photostimulation with a single laser pulse produced a fast change in IPSC activity. Bottom, tetanus photostimulation at the same location increased IPSC activity over many seconds. A cluster of burst of IPSCs is identified by the asterisk in the trace. Scale 20 pA and 50 ms. D, Raster plot showing the responses to a single and subsequent train of laser pulses for the cell shown in B. Marks indicate IPSC activity 2xSD above the level of baseline activity Bins = 1 sec. E, Raster plot showing the responses to a single and subsequent train of laser pulses for the cell shown in C. F, Population data (mean ± standard error) illustrating IPSC frequency over time for a single laser pulse. The blue shadow indicates the onset of the stimulation. Bins = 1 sec. G, Population data (mean ± standard error) illustrating IPSC frequency over time for a tetanus photostimulation.
Figure 26: mGluRs regulate prolonged inhibitory output from Type-B F2 terminals. 

Ai, Shown is the baseline IPSC activity while in TTX (1uM). Aii, Tetanus photostimulation at increased IPSC activity over many seconds. Aiii, Application of MPEP (50 µM) attenuated the duration of IPSC activity. Aiv, Subsequent application of DNQX (20-40 µM) blocked the initial fast change in IPSC activity. Av, Raster plot showing the responses to a train of laser pulses for the cell shown above, before and after MPEP application. B, Population data (mean ± standard error) illustrating IPSC frequency over time for the two conditions (1 sec bins).
4.6.0. References


Chapter 5

Conclusion

5.0.0. Concluding remarks

This dissertation has examined how the dendrites of inhibitory thalamic neurons integrate excitatory synaptic potentials. Using new electrophysiological and imaging techniques, work performed in this dissertation clearly demonstrates that the distal dendritic regions of these neurons have unique physiological properties that transform synaptic input into neuronal output. Although the relationship between dendrites and higher-level function remains speculative, it is likely that these unique properties are important determinants of how inhibitory thalamic neurons generate output during normal thalamocortical activities. Overall, this dissertation includes three major findings with regards to inhibitory thalamic neurons. First, in thalamic reticular neurons, distal dendrites contain voltage-gated T-type calcium channels which can amplify or boost distal inputs, thereby ensuring integration at the somatic level (Chapter 2) (Crandall et al., 2010). Second, in thalamic interneurons, release of GABA from presynaptic dendrites is regulated by ionotropic glutamate receptors, thereby allowing dendritic release to occur in response to local excitatory inputs (Chapter 3) (Crandall and Cox, 2012). Finally, the presynaptic dendrites of thalamic interneurons contain two distinct types of terminals based on glutamate receptor regulation, thereby enabling interneurons to regulate relay neuron excitability through two distinct feedforward inhibitory pathways (Chapter 4).

The physiological data presented in chapter 2 clearly indicate, in thalamic reticular neurons, a high density of T-type voltage-gated calcium current ($I_T$) is located in the distal
dendrites of these neurons. These data are consistent with previous computational work which predicted that high densities of I_T must be located in distal dendrites to account for the unique bursting pattern observed in TRN neurons (Destexhe et al., 1996). Moreover, the data presented demonstrate that distal I_T can amplify local inputs, independent of soma/proximal I_T. Based on these findings, we hypothesize that the voltage-state of dendritic T-type calcium channels would be an important determinate of how TRN neurons integrate afferent information across space. For example, if the somatodendritic axis was uniformly hyperpolarized, sufficient distal input would activate local T-type calcium channels which, in turn, would generate a local calcium spike that propagates actively and reliably toward the soma. In this scenario, distal input would result in robust output from TRN neurons, in the form of a burst discharge. Alternately, if the proximal dendrites were relatively depolarized compared to the distal dendrites, distal signals would still be amplified considering activation of T-Type channels. However, the output would be less robust since the resulting potential at the soma would be smaller in magnitude due to attenuation caused by the passive membrane properties of the dendrites. Lastly, if the distal dendrites were sufficiently depolarized, so to inactivate local T-type calcium channels, input could not be amplified, thereby disconnecting distal dendrites from communicating information to the soma/axon. If this hypothesis is true, it would allow TRN dendrites to transform input into distinct outputs depending on the state of the dendrites. In theory, this state-dependent dendritic integration would also be branch specific, enabling individual dendrites to behave in independent of each other.

These results are also intriguing if we consider the functional architecture of the TRN (Guillery and Harting, 2003). The TRN is located at the interface of the thalamus and neocortex, where it receives excitatory input from both structures. As these axon fibers traverse the TRN
they give rise to dense zones of terminal innervation. These zones occupy distinct sectors of the
TRN, with each sector innervated by merging axon terminals of associated thalamic and
neocortical areas. Furthermore, there is convincing anatomical data to suggest that each TRN
sector can be further divided into 2-3 components called slabs. Slabs lie parallel to the plan of the
TRN and have been show to receive input from discrete sub-regions of the associated areas.
Because TRN neurons possess elongated dendrites, borders between sectors are not identifiable
without labeling the afferent fibers. This suggests that at least some TRN neurons would have
dendrites that cross into neighboring sectors and or slabs. We propose that distal TRN dendrites
could serve as sites of state-dependent integration within and across different functional
modalities. Such actions would be dependent on not only the level of afferent activity but the
voltage-state of the somatodenritic axis. In theory, such a mechanism could facilitate focused
thalamocortical communication by suppressing activity in neighboring thalamocortical pathways
(i.e., intra- or cross-modality).

In order to validate this hypothesis, future studies will need to be performed to verify the
predictions of this model. First, we need to identify key neuromodulators that could alter the
voltage-state of TRN dendrites and, in turn, synaptic integration. I would propose to determine
the effect of specific neuromodulators on dendritic excitability, and thereby their influence on
state-dependent synaptic integration in TRN neurons. My working hypothesis is that certain
neuromodulators located within the thalamocortical circuits serve as endogenous modulators that
are released in either an activity-dependent or state-dependent manner, thereby altering dendritic
excitability. I would focus on two specific neuromodulator systems targeting the TRN:
cholinergic and cholecystokinin. Previous work has shown that cholinergic and cholecystokinin
input produces long-lasting changes in TRN excitability by increasing or decreasing a potassium conductance, respectfully (Cox et al., 1995; McCormick and Prince, 1986).

Second, we need to identify the degree to which individual TRN neurons receive cross-modal information. I would propose using a virus to express channelrhodopsin-2, a light-sensitive cation channel, in pathway-specific circuits so to selectively stimulation their axons and record their responses in TRN neuron in vitro (Cruikshank et al., 2010; Petreanu et al., 2007; Zhang et al., 2006). For example, labeling layer V pyramidal neuron in the auditory cortex and recording responses in TRN neurons that project to the ventrobasal nucleus (i.e., somatosensory sector). My working hypothesis is that certain TRN neurons, located near the border of two sectors, serve as cross-modal integrators that are capable of responding, in an activity-dependent manner, to input from two distinct sensory cortices. I would focus on modalities that have sectors neighboring one another within the TRN: visual and auditory, auditory and somatosensory, or somatosensory and motor. Previous work in anaesthetized animals has shown some TRN neurons can respond to more than one modality (Shosaku and Sumitomo, 1983; Sugitani, 1979).

The data presented in chapter 3 and 4 focused on the presynaptic dendrites of thalamic interneurons. This work clearly demonstrates that the presynaptic terminals found within these dendrites are capable of producing inhibitory output in response to local excitatory input. This provides convincing evidence that thalamic interneurons are capable of behaving as multiplexing integrators, with numerous independently operating input-output devices. Our work is consistent with previous computational work which predicted, based the electrophysiological and morphological properties, that presynaptic dendrites would operate independent of the soma/axon because interneurons are highly non-compact electrotonically (Bloomfield and Sherman, 1989). Moreover, we demonstrate that there are at least two distinct types of
dendrodendritic synapses, formed between interneurons and thalamic relay neurons, based on their glutamate receptor regulation. This novel finding indicates that two distinct feedforward inhibitory pathways, involving inhibitory presynaptic dendrites, exist in the thalamus. In the case of the visual thalamus, we hypothesize that these pathways regulate distinct retinoganglion cells that not only project to the same thalamic relay neuron but encode different aspects of visual space. Although the involvement of the retinogeniculate pathway remains speculative, anatomical and physiological data strongly suggests this pathway is directly involved in dendrodendritic communication in the visual thalamus (Sherman, 2004).

To test this hypothesis, future studies will need to be performed. First, we need to determine if retinogeniculate terminals locally drive both types of dendrodendritic synapses. I would propose to use a virus to express channelrhodopsin-2 in retinoganglion cells, so their axon terminal can be selectively stimulated to drive (locally) the presynaptic dendrites of thalamic interneurons. My working hypothesis is that retinogeniculate terminals serve as endogenous drivers of both types of dendrodendritic terminals identified. Furthermore, optogenetic techniques could be used to selectively express channelrhodopsin-2 (excitatory) or halorhodopsin (inhibitory) in thalamic interneurons (Yizhar et al., 2011). Using this approach we could investigate the function of thalamic interneuron and their presynaptic dendrites during natural visual processing in vivo.
5.1.0. References


