ACTIVITY-DEPENDENT SYNAPTIC PLASTICITY WITHIN RAT THALAMOCORTICAL CIRCUITRY

By

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ABSTRACT

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Thalamocortical circuits are involved in the transfer of sensory information to the cortex, as well as corticocortical communication. Information transfer through thalamocortical circuits is dynamically regulated by intrathalamic circuitry and external afferents (e.g. corticothalamic and brainstem inputs), but the degree to which synaptic plasticity occurs in the thalamus is not well characterized. I sought to investigate the role of activity-dependent synaptic plasticity in thalamic neurons. Electrophysiological recordings were obtained from thalamocortical relay neurons in the dorsal lateral geniculate nucleus (dLGN), ventrobasal nucleus (VB), and neurons in the thalamic reticular nucleus (TRN) using *in vitro* slice preparations.

I first address the role of state-dependent synaptic plasticity by determining the influence of thalamic action potential firing modes on plasticity. Thalamic neurons ubiquitously fire in either burst or tonic firing modes, which are mediated by voltage-dependent T-type Ca^{2+} channels. Since the firing mode can change based on an organism's behavioral state, findings regarding the role of plasticity with respect to burst and tonic firing will be valuable in determining how sensory information transfer is modulated. I show that thalamic relay neurons display short-term alterations in excitatory synaptic responses following postsynaptic pairing (i.e., burst or tonic firing) paired with corticothalamic activation. The magnitude and frequency of occurrence of short-term depression or facilitation differed depending on the firing state of the postsynaptic neuron.

This plasticity was not observed when primary sensory afferents (retinogeniculate fibers) were stimulated, indicating the changes observed are restricted to corticothalamic inputs. TRN neurons, display depression following paired burst firing, which may affect the way in which the TRN mediates relay neuron activity in the thalamocortical circuit.

When unpaired tetanic stimulation of corticothalamic afferents is applied, most thalamic neurons display short-term facilitation, irrespective of firing mode. I next elucidated the mechanism of activity-dependent short-term plasticity in thalamic relay neurons. I show that short-term facilitation is Ca^{2+}-dependent and mediated by the adenylyl cyclase pathway. Adenylyl cyclase activation and cAMP production could lead to downstream modifications in ion channels. Blocking K^+ currents and inhibiting hyperpolarization-activated cyclic-nucleotide modulated currents attenuated the tetanus-induced facilitation, indicating the dependence of K^+ channels and the hyperpolarization-activated current (I_h) to this plasticity. I hypothesize that short-lasting alterations in these currents leads to the observed enhancement in neurotransmitter release and facilitation of the postsynaptic response following tetanic stimulation.

Novel findings regarding synaptic plasticity in the thalamocortical circuit can have major implications on the role of cortical feedback on information transfer through the thalamus to the neocortex. Enhancing excitatory activity at corticothalamic synapses can influence the relay of sensory information to the cortex, increase thalamic throughput, and potentially impact the nature of sensory information.

This work is dedicated to my parents, who always inspired me to work hard for my dreams.

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KEY TO ABBREVIATIONS

2PLSM	2-Photon laser scanning microscopy
AChR	Acetylcholine receptor
AMPAR	AMPA receptor
CI	Confidence interval
CT	Corticothalamic
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
D	Dark synapse
dLGN	Dorsal lateral geniculate nucleus
DRN	Dorsal raphe nucleus
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
F	Flat synapse
IPSC	Inhibitory postsynaptic current
IC	Internal Capsule
LTD	Long-term depression
LTP	Long-term potentiation
I_T	Current through T-Type Ca^{2+} channels
LTS	Low-threshold Ca^{2+} spike
MGN	Medial geniculate nucleus
mGluR	Metabotropic glutamate receptor
NMDAR	NMDA receptor
NO	Nitric oxide

KEY TO ABBREVIATIONS (cont'd)

OD	Outer diameter
P	Pale synapse
PBR	parabrachial region
PKA	protein kinase A
PKC	Protein kinase C
PP1	Protein phosphatase 1
PTP	Posttetanic potentiation
R	Round synapse
RG	Retinogeniculate
RMP	Resting membrane potential
S	Small synapse
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
STDP	Spike-timing-dependent plasticity
TC	Thalamocortical
TRN	Thalamic reticular nucleus
VB	Ventrobasal nucleus
V_{hold}	Holding potential
V_m	Membrane voltage
V1	Primary visual cortex
V2	Secondary visual cortex
vLGN	ventral lateral geniculate nucleus

CHAPTER 1: INTRODUCTION

Thalamocortical circuits are traditionally known for their contribution to sensory signaling, but they are also involved in alterations of behavioral states (i.e. sleep vs. wake, attention, arousal), as well as pathophysiological conditions, such as aberrant sensory integration and epilepsy (Gloor P., 1984; Steriade et al., 1993; Steriade and Contreras, 1995; Sillito and Jones, 2002; Cappe et al., 2009; Tyll et al., 2011). While the thalamus was originally thought to function as a direct relay to transfer unaltered information from sensory inputs to the neocortex, it is now evident that information modification occurs within the thalamocortical circuit (Krupa et al., 1999; Sillito and Jones, 2002; Sherman, 2007; Varela, 2014), and can be modulated by intrathalamic connectivity, external afferents (corticothalamic and brainstem afferents), and intrinsic properties of thalamic neurons (Bal et al., 2000; Destexhe, 2000; Cox, 2014; Varela, 2014).

Thalamic relay neurons receive information from primary sensory afferents, and transfer information to respective cortical regions. Deep layer corticothalamic neurons reciprocally connect with the thalamus, and this feedback pathway makes up the majority of inputs onto thalamocortical relay neurons (Sherman and Koch, 1986; Liu et al., 1995; Erisir et al., 1997b; Van Horn et al., 2000), suggesting that corticothalamic afferents have a strong influence on controlling thalamic neuronal activity (Sherman and Koch, 1990). Synaptic plasticity at corticothalamic synapses is of particular interest, because plasticity may be one mechanism by which the cortex can exert fine synaptic control on thalamic neurons, to produce a lasting influence sensory information transfer. While some studies have explored synaptic plasticity and its implications in the thalamocortical circuit, the degree to which state-dependent and short-term excitatory synaptic plasticity occurs in the

thalamus is not well characterized. Short-term plasticity, which is an alteration in synaptic efficacy lasting less than 20 minutes, could significantly affect the way in which sensory information is relayed to the cortex. A short-lasting alteration in synaptic efficacy at the thalamic level could represent a mechanism for dynamically altering thalamic throughput to the cortex. Additionally, synaptic plasticity could represent a mechanism through which cortico-cortical communication of sensory information is facilitated via the thalamus. By understanding the mechanism of short-term plasticity, and how it can impact sensory information transfer, we will gain a greater understanding of the different ways that information can be conveyed or modified through the thalamocortical circuit.

The long-range goal of this research is to characterize synaptic plasticity in different thalamic nuclei, and explicate the role of synaptic alterations in the thalamocortical circuit. There have been a limited number of studies examining plasticity in thalamocortical circuits, and new findings will not only allow us to understand synaptic modulation at thalamic synapses, but also gain insight regarding sensory processing and potentially dysfunctional thalamic plasticity in pathophysiological conditions.

1.1 Functional anatomy of the thalamus

The thalamus is made up of three major brain regions: epithalamus, dorsal thalamus, and ventral thalamus. The epithalamus is made up of the paraventricular and habenular nuclei, and generally does not send nor receive inputs from the cortex (Jones, 1985). Our focus will be on thalamic nuclei from the dorsal and ventral thalamus. (Herrick, 1918; Clark, 1932; Droogleever-Fortyun, 1950).

The dorsal thalamus can be organized into different nuclei based on the anatomical connections and sources of information that are transferred to the cortex. The dorsal

thalamus has been differentiated into nuclei of three types: relay nuclei, the association nuclei, and the midline and intralaminar nuclei (Jones, 1985; Price, 1995). The relay nuclei receive sensory or motor information through ascending pathways, and send it to the primary sensory areas of the cortex. Relay nuclei include the lateral geniculate nucleus, medial geniculate nucleus, ventral posteromedial and posterolateral nuclei, posterior nucleus, ventral lateral nucleus, ventral anterior nucleus and the ventral medial nucleus. Association nuclei do not receive primary sensory afferents, but receive projections from layer 5 pyramidal cells of primary cortical regions and send information to association areas of the cortex. The intralaminar nuclei send projections to both subcortical and cortical regions, and are thought to be involved in arousal and attention (Powell and Cowan, 1954; Cowan and Powell, 1956; Francois et al., 1991; Gimenez-Amaya et al., 1995). These studies will primarily focus on the primary sensory relay nuclei of the dorsal thalamus.

A. Dorsal Thalamus

The primary sensory relay nuclei of the dorsal thalamus include the dorsal lateral geniculate nucleus (dLGN), which relays visual information; the ventral posterior nucleus (VB), which transfers somatosensory information; and the medial geniculate nucleus (MGN), which sends auditory information. Since the dLGN and its connections have been well characterized, it will be used as the model relay nucleus for examples of thalamocortical circuitry. Most of the circuit features that will be covered in the dLGN can be further generalized to the VB and MGN.

B. Ventral Thalamus

The ventral thalamus is distinct from the dorsal thalamus, in that it does not send any axons to the cortex. The ventral thalamus is composed of the thalamic reticular nucleus (TRN), ventral lateral geniculate nucleus (vLGN), zona incerta, and nucleus of the field of Forel (Jones, 1985). The ventral thalamus is involved in some visually related movements. The TRN, zona incerta, and vLGN all send projections to multiple nuclei in the dorsal thalamus. The TRN is strategically located around the dorsal thalamic nuclei, and provides GABAergic inhibition to these neurons (Hendrickson et al., 1983; Penny et al., 1983; Jones, 1985; Rinvik et al., 1987; Smith et al., 1987; Bentivoglio et al., 1991; Arcelli et al., 1997; Dixon and Harper, 2001). The TRN will also be tested in these experiments, since we will be determining the role of corticothalamic synaptic plasticity on TRN neurons, and the influence of TRN inhibition on relay neurons.

1.2 Organization of the thalamocortical circuit

To understand the contribution of synaptic plasticity in different synapses within the thalamus, it is necessary to understand the organization of the thalamocortical circuit (**Fig. 1.1**) and the different afferents to relay nuclei (**Fig. 1.2**).

A. Sensory inputs

Primary sensory afferents transfer the main information from each sensory modality to its respective relay nucleus. Visual information is initially processed in the retina, and ganglion cells, which are the output neurons of the retina, send axonal projections to the dLGN. (Guillery, 1969b). Retinal inputs form synaptic contacts primarily on proximal dendrites of relay neurons (Erisir et al., 1997a) (Guillery, 1969a). dLGN relay

neurons then send projections, known as thalamocortical afferents, primarily to layer 4 of the visual cortex (V1 and V2).

The other sensory relay nuclei receive inputs from their respective primary sensory afferents. The VB receives sensory information via the medial and trigeminal lemnisci and the spinothalamic tract, which convey somatosensory information to relay neurons. Thalamocortical afferents from the VB project to cortical regions S1 and S2. The MGN receives primary auditory inputs from the inferior colliculus and projects to cortical regions A1 and A2, the temporal auditory field, and diffuse insular-temporal/parietal regions (Jones, 1985).

B. Cortical inputs

The neocortex sends glutamatergic feedback projections to the thalamus. Synaptic inputs to relay nuclei are mainly dominated by these corticothalamic feedback projections. Corticothalamic projections make up 30% of incoming afferents to the LGN (Weber and Kalil, 1987; Sherman and Koch, 1990; Montero, 1991; Erisir et al., 1997b; Van Horn et al., 2000). These corticothalamic projections form contacts primarily on distal dendrites of relay neurons (Hamos et al., 1985; Montero, 1991; Liu et al., 1995).

The thalamocortical circuit is especially unique, in that every dorsal thalamic nucleus that sends axonal projections to the cortex, also reciprocally receives corticothalamic feedback afferents (Diamond et al., 1969; Jones, 1985). However, it is important to note that the degree of reciprocity is not necessarily equivalent. In fact, corticothalamic afferents outnumber thalamocortical axons by 10:1 (Sherman and Koch, 1986; Deschenes et al., 1998).

Corticothalamic feedback afferents project to a location-specific region of the relay nucleus that activated it. This interesting feedback organization shows that corticothalamic projections directly excite the same group of neurons that initially activated it (Temereanca and Simons, 2004; Li and Ebner, 2007). Additionally, the corticothalamic pathway can indirectly inhibit relay neurons via activation of the TRN (Lam and Sherman, 2010) (Sillito et al., 2006). These features demonstrate the capabilities of corticothalamic feedback afferents to modulate information transfer through the circuit.

Several cortical lesion experiments detail the direct mapping of cortical projections to specific regions of relay nuclei. Smaller cortical lesions produce specific segments of rapid neuronal degeneration in the respective region of the relay nucleus that it receives projections, while larger lesions produced dramatic retrograde degeneration in most of the thalamus (Monakow, 1889; Nissl, 1913). These experiments show how the cortex provides feedback projections to localized regions of the thalamus, and how the loss of large cortical regions can affect the survival of relay neurons. Interestingly, destruction of the whole cerebral cortex produced degeneration in all thalamic nuclei, except for the TRN, epithalamus, and the intralaminar nucleus (Dubin and Cleland, 1977; Ahlsen et al., 1982; Lindstrom and Wrobel, 1990) . This evident difference in survival between relay neurons and TRN demonstrates the reliance of relay neurons on the cortex, while TRN neurons are able to survive in the absence of the cortex.

C. Non-cortical inputs

When all non-retinal inputs are combined, these form up to 90% of afferents to the dLGN (Sherman and Koch, 1990) (**Fig. 1.2**). Other major non-cortical inputs include the brainstem projections, which account for up to 30% of inputs to the LGN (Cucchiaro et al.,

1988; Hallanger and Wainer, 1988; Fitzpatrick et al., 1989). The LGN receives cholinergic inputs from the parabrachial region (PBR), noradrenergic afferents from the PBR and locus coeruleus (Nakamura et al., 1984; Morrison and Foote, 1986; De Lima and Singer, 1987), serotinergic afferents from the dorsal raphe nucleus (DRN) (De Lima and Singer, 1987; Kayama et al., 1989; Gonzalo-Ruiz et al., 1995), and histaminergic projections from the tuberomammillary nucleus (Uhlrich et al., 1993).

The LGN, specifically, receives GABAergic inputs from the pretectum (Cucchiaro et al., 1991), cholinergic inputs from the parabigeminal nucleus of the midbrain (Harting et al., 1991), and superior colliculus afferents, which all influence visual information processing.

D. Intrathalamic inputs

Relay nuclei receive afferents that originate within the thalamus, as well. While thalamic relay neurons have few direct connections to each other, local interneurons can provide GABAergic inhibition to relay neurons. In the rodent, interneurons are only present in significant numbers in the dLGN (Arcelli et al., 1997). Interestingly, relay neurons can directly excite interneurons, which in turn, provide feedback inhibition back onto relay neurons (Sanchez-Vives et al., 1996; Cox et al., 2003; Bickford et al., 2008), which may be a mechanism through which relay neurons can indirectly control their own activity.

The TRN also provides direct GABAergic afferents to relay nuclei (Scheibel and Scheibel, 1966; Jones, 1985; Yen et al., 1985; Uhlrich et al., 1991; Pinault et al., 1995; Cox et al., 1996). While all TRN neurons are GABAergic, there is evidence that there may be different types of TRN neurons in terms of morphology, spike discharge properties, and inhibitory drive onto relay nuclei (Cox et al., 1997; Lee et al., 2007), which could impact the

degree to which TRN neurons are able to modulate relay neuron activity. It has been demonstrated (in cat and rat) that TRN neurons form connections with each other within the nucleus itself (Scheibel and Scheibel, 1966; Deschenes et al., 1985; Jones, 1985; Yen et al., 1985; Spreafico et al., 1988; Steriade and Llinas, 1988; Cox et al., 1997; Sherman and Guillery, 2006). The ability of the TRN to synchronize during rhythmic activity has been attributed to these local axonal, dendrodendritic, and electrical connections (Deschenes et al., 1985; Pinault et al., 1997; Landisman et al., 2002).

E. Morphological structure of afferents

The various afferents to relay nuclei have different dendritic and synaptic structures, which contribute to their particular role in transmitting information to relay neurons. Primary sensory afferents display thick, richly branching axons (Jones, 1983; Bowling and Michael, 1984; Sur et al., 1987). These axons were identified as "Type II" in the cat, by Guillery (1969). Non-sensory inputs have smaller, more scattered side branches. These inputs send collaterals to the TRN, and are classified as "Type I" axons (Guillery, 1966).

The presynaptic structure of the terminal that synapses onto relay neurons can also be used to distinguish afferents based on terminal contents, synaptic function, extrathalamic origin, and neurotransmitter used. Sherman and Guillery (2004) formed an interpretation of thalamic inputs, which introduces their distinction between "drivers" and "modulators". Additionally, electron micrograph experiments distinguish the synaptic structure of afferents based on the driver and modulator distinctions. Afferents can be differentiated, using electron micrograph imaging, as drivers or modulators based on vesicle shape or terminal size. The shape of the vesicle is determined by its synaptic

contents, and can be either round (R) or flat (F). R type synapses are usually glutamatergic, while F type synapses are typically GABAergic. Synaptic terminal size can be either large (L) or small (S). Lastly, mitochondria color is used to classify synapses as either pale (P) or dark (D), but the functional correlate of color is not well characterized

Driver inputs to relay nuclei come from primary sensory afferents (i.e. retinal ganglion cell axons to the dLGN). Driving pathways bring information about the environment to the major thalamic nuclei via sensory pathways (e.g. visual, auditory, somatosensory, taste), or subthalamic centers (cerebellum, mammillary bodies), and determine the qualitative characteristics of sensory receptive fields. For example, each retinal ganglion cell that makes up the driving afferents to the LGN carries visual information from a specific region of the visual field, which makes up the receptive field for that area of space. Retinal ganglion cell afferents transfer information (via center-surround visual receptive field organization) to the LGN and subsequently to the visual cortex, where the representation of the visual field can be perceived (Sherman and Guillery, 2006). Additionally, driving inputs to thalamic relay nuclei innervate both relay neurons and interneurons, but not TRN. Typically, driver afferents are observed in electron micrograph images as RLP. They are usually seen in glomeruli, where they synapse onto F profiles, to form triadic synapses (discussed below) (Szentagothai, 1963; Colonnier and Guillery, 1964; Peters and Palay, 1966; Guillery, 1969a).

Other inputs to the relay nuclei are known as “modulators,” which affect the quantitative aspect of the receptive field, rather than its qualitative structure. For example, modulators can affect the size of the receptive field, or the firing rate of relay neurons. Experiments have shown that silencing a driver produces a loss of receptive field, while

silencing a modulator causes the receptive field to persist (Kalil and Chase, 1970; Schmielau and Singer, 1977; Geisert et al., 1981; Yuan et al., 1985; Diamond et al., 1992). These studies demonstrate how loss of driving inputs can abolish the receptive field altogether, which emphasizes the importance of drivers in maintaining the receptive field properties. Modulators form contacts with relay and interneurons, and they also send collaterals through the TRN (Jones, 1985; Sherman and Guillery, 2002). Modulators are represented by RSD synapses, with the majority originating from the cortex, and some coming from the brainstem (Erisir et al., 1997a). These synapses are usually extraglomerular, and have smaller terminals, with closely packed vesicles. The F profiles make up modulatory GABAergic synapses onto relay neurons (Gray, 1969). F1 terminals come from the TRN, interneurons, or other extrinsic GABAergic afferents. F2 terminals come from the dendrites of interneurons, and form the unique triadic circuitry with retinogeniculate synapses and relay neuron dendrites (Liu et al., 1995; Lam et al., 2005).

F. Classification of thalamocortical neurons

Thalamocortical relay neurons have been differentiated into multiple subtypes based on morphological and functional criteria. The differing dendritic arbor, axon diameter, and soma size can all impact the flow of information through a the thalamocortical circuit. Differences in intrinsic properties of neurons can also paly a role in neuronal response to integration of afferent signals. These factors all contribute to the integration of incoming information in relay neurons. Synaptic integration will become especially important regarding synaptic plasticity, since the nature of synaptic inputs has the potential to change the relay neuron's ability to transfer information to the cortex.

Thalamic relay neurons can be classified based on dendritic arbor and soma size, as first proposed by Kolliker (1896), where he identified relay neurons (*Buschzell*), interneurons, and reticular neurons (*Strahlenzell*). A later reassessment by Friedlander et al. (1981) of Kolliker's original classifications led to a new identification system based on both function and physiology of relay cells, which are now called X, Y, and W type cells (Friedlander et al., 1981). In the cat, bushy cells can be functionally identified as "X-type" cells, while radiate cells are referred to as "Y–type" cells. The dendritic arbor of X cells are tufted, elongated and oriented perpendicularly to the plane of the laminae; Y cell dendrites have a stellate distribution with a spherical arbor. Additionally, X cells specifically display glomeruli, which is the location of interneuronal contacts indicative of F2 circuitry (Szentagothai, 1963; Guillery, 1966; Friedlander et al., 1981; Stanford et al., 1983; Lam et al., 2005).

Y cells are more prevalent than other relay cell types. Y cells also have larger receptive fields, faster conducting axons, better responses to visual stimuli and low spatial/high temporal frequencies, but poor responses to high spatial/low temporal frequencies. Y cells respond to higher spatial frequencies with a nonlinear response, whereas X cells demonstrate linear summation to stimuli (Sherman and Spear, 1982; Shapley and Lennie, 1985; Sherman, 1985). One last type of relay cell type was identified, and it was unlike the bushy or radiate groups. This type is called the W cell, and is smaller in size than the other two types (Guillery, 1966; Stanford et al., 1983).

Similar to Friedlander's classifications, Krahe et al. (2011) showed a comparable morphological distinction in dLGN relay neurons of mice, which he called X-like, Y-like, or

W-like cells (Krahe and Guido, 2011). The size and percentage of each cell type was similar to that seen in the cat, but the cells did not show any physiologically different properties.

G. Parallel pathways in the dLGN

It has been shown that there are different parallel pathways from retinal ganglion cells to relay neurons, and to the cortex. Parallel pathways are independent channels for transmission from retinal ganglion cell through to the visual cortex, with the distinctions based on retinal ganglion cell type. A key element of parallel pathway organization is the presence of separate cell types at all levels of processing (Alexander et al., 1986).

The three functionally different relay cells in the primate and cat LGN each receive distinct axons from a specific type of retinal ganglion cell, and terminate in different laminar layers of the cortex. These cells are characterized by different cell body sizes and dendritic arbors and in some species, there is a characteristic distribution within the laminae of the LGN.

In the cat independent parallel pathways are observed, which project to the cortex. The on-center and off-center pathways, which determine how retinal ganglion cells and relay neurons fire in response to a visual stimulus, are both present in the A laminae (Rodieck, 1998), while the W cells are in a separate layer.

In the primate, there is some separation of parallel pathways, where on-center relay neurons are partially separated from off-center relay neurons in distinct geniculate layers. However, this separation is only evident for central vision.

In the rodent, the presence of parallel organization in the LGN has not been clearly determined. Krahe et al. (2011) showed regional preferences of the X-like, Y-like, and W-like relay neurons throughout the nucleus. They showed that X-like cells were mainly

present in the ventral part of the nucleus and contained monocular projections, Y-like cells were present in the central core of the nucleus, and contained ipsilateral projections, and W-like cells were found along the perimeter of the nucleus. However, it is not known whether these morphological features correlate with the functional features, seen in the cat. Studies from the rat show that the core region includes cells that receive input from fast-conducting, large, type I RGCs, while the surrounding region receives input from slowly conducting, smaller type II and III RGCs, and a potential third region that receives monocular inputs from smaller type II and III RGCs. However, the morphological correlates of these physiological features in the rat has not been determined (Martin, 1986; Reese, 1988). These patterns of relay cell distribution in different species demonstrate that laminar segregation of relay cells is not necessary to keep the distinct parallel pathways separate.

1.3 Intrinsic cellular properties of thalamic neurons

A. Physiological ramifications of relay and TRN neuronal morphology

There are evident morphological differences between relay and TRN neurons that affect how these neurons physiologically integrate and process incoming information. Relay neurons (**Fig. 1.3A**) are electrotonically compact, meaning voltage changes applied at distal locations will attenuate by less than half at the level of the soma. Relay neurons are electrotonically coupled due to the uniform thickness of their dendrites; thicker dendrites allow more current from synaptic activation to flow through the cytoplasm and to the soma, leading to less current leak through the membrane (Crunelli et al., 1987b; Bloomfield and Sherman, 1989; Sherman and Guillery, 2006). These dendritic properties of thalamic relay neurons lead to efficient current flow, with low dendritic attenuation; therefore, most

active synapses to relay neurons will substantially influence the axon hillock, leading to significant levels of synaptic integration and summation (Sherman and Guillery, 2006).

Contrastingly, TRN neurons (**Fig. 1.3B**) are more electrotonically extensive, since they have long bipolar dendrites, making the cell less compact. Due to the long dendrites of TRN neurons, there is the potential for current loss due to passive cable properties. As a compensatory mechanism for potential loss of current, TRN dendrites actively conduct potentials towards the soma via strong active conductances, rather than passively propagating potentials. These strong active dendritic conductances can act to boost excitation from distally-synapsing inputs, thereby effectively transferring inputs to the soma. Crandall et al. (2010) showed that distal dendritic Ca^{2+} currents from T-type Ca^{2+} channels (I_T) in TRN neurons may act to amplify afferent inputs. Additionally, while relay neurons also show active dendritic conductances due to I_T, the large magnitude at distal dendrites, compared to proximal dendrites, is much greater than in relay neurons. A greater dendritic response can ensure integration of synaptic inputs at the somatic level by compensating for attenuation, especially in the long, extensive TRN dendrites (Crandall et al., 2010; Errington et al., 2010). All of these features could be possible mechanisms to compensate for the electrotonic limitations of TRN neuronal morphology.

B. Burst vs. tonic firing modes

Virtually all thalamic neurons fire action potentials in two distinct states, known as tonic or burst firing modes (McCarley et al., 1983; Deschenes et al., 1984; Jahnsen and Llinas, 1984a, c; McCormick and Huguenard, 1992). Generally, tonic mode mainly occurs during brain-activated states, such as wakefulness, attentiveness, and REM sleep. Burst mode is present during synchronized slow waves, such as during slow-wave sleep,

anesthesia, and a variety pathophysiological conditions, including absence seizures (Hirsch et al., 1983; McCarley et al., 1983; Fourment et al., 1985; Gloor and Fariello, 1988; Buzsaki et al., 1990; Steriade et al., 1990c). However, it is important to note that both firing modes have been observed in awake animals (Guido and Weyand, 1995; Nicolelis et al., 1995; Ramcharan et al., 2000), suggesting that burst mode does may play a distinct role in sensory information processing, which will be discussed below.

B1. I_T mediates firing mode

Burst firing is mediated by the activation of I_T, allowing Ca^{2+} influx to occur through voltage-dependent T-type Ca^{2+} channels located on the soma and dendrites of thalamic neurons (Jahnsen and Llinas, 1984b; Destexhe et al., 1998). The T-type Ca^{2+} channel has three different states: deinactivated, activated, or inactivated, and has both a voltage and time dependency. T-type Ca^{2+} channels are deinactivated at hyperpolarized potentials and following a depolarizing stimulus, activation of I_T occurs. During membrane depolarization, T-type Ca^{2+} channels become inactivated, preventing any further influx of Ca^{2+}. I_T becomes activated at ~-75 mV and becomes progressively inactivated at more depolarized potentials, with complete inactivation occurring at ~-60 mV (Jahnsen and Llinas, 1984a, c; Suzuki and Rogawski, 1989).

At hyperpolarized membrane potentials (< -70 mV), burst discharge will be the response following a suprathreshold stimulus. Ca^{2+} influx through activated T-type Ca^{2+} channels produces a low-threshold Ca^{2+} spike (LTS), which brings the membrane to action potential threshold, leading to a transient, high frequency burst of action potentials on top of the LTS. I_T has a shorter time constant for activation compared to inactivation or deinactivation, which takes ~100 ms (acting like a refractory period, and preventing

another stimulus from activating the current). This long duration for deinactivation limits the burst firing rate to 10 Hz or less. Therefore, thalamic neurons need a moderate rate of depolarization to activate I_T; if the rate of depolarization is too gradual, this will initiate a slower membrane depolarization, inactivating T-type Ca^{2+} channels, and will switch the cell to tonic firing mode (Sherman, 2001a).

When I_T is inactivated by membrane depolarization (~-55 mV), the cell will respond to a suprathreshold input with tonic action potential firing (See **Figure 1.4**). I_T is ubiquitous in relay neurons of the dorsal thalamic nuclei, meaning most thalamic neurons fire in burst or tonic modes (Deschenes et al., 1984; Jahnsen and Llinas, 1984a; Hernandez-Cruz and Pape, 1989; McCormick and Feeser, 1990; Scharfman et al., 1990; Bal et al., 1995).

Based on analysis of the firing mode and ability to encode and transfer visual information, it has been shown that both firing modes can convey equal amounts of information to the cortex (Reinagel et al., 1999). However, the firing modes transfer the *nature* of incoming information in different ways.

During tonic firing, the frequency of action potential discharge correlates linearly with the duration and intensity of the stimulus (i.e. a less salient stimulus evokes a lower firing frequency) (Zhan et al., 1999). Additionally, tonic firing produces action potentials that show less spike-frequency adaptation, which allows cells in tonic mode to faithfully transmit sensory information without much reduction in firing rate during prolonged stimulation. Therefore, tonic firing is representative of a linear transformation of afferent signals to the cortex.

Burst mode, on the other hand, does not accurately reflect the strength or the duration of the incoming stimulus (Guido and Weyand, 1995; Zhan et al., 2000; Sherman,

2001b). A suprathreshold stimulus will elicit an LTS, which does not accurately reflect the properties of the stimulus (Zhan et al., 1999). Another prominent feature of burst mode is that it displays a greater signal-to-noise ratio than tonic mode. In burst mode, lower spontaneous activity (noise) is evident, so the visual response (signal) is greater than in tonic mode, which displays more spontaneous activity (Guido et al., 1995). These features indicate that burst mode may be better capable of initial stimulus detection, due to its strong ability to activate the cortex (Swadlow and Gusev, 2001).

B2. Burst discharge maximizes novel stimulus detection

Burst mode has been implicated in initial detection of a new stimulus, while tonic mode presides during awareness and wakeful states, due to the nature of information transfer (Steriade et al., 1986; Steriade et al., 1990a; Guido et al., 1995; Sherman, 2001a). Isolated burst firing may contribute to the emphasis or transmission of important sensory features, since burst discharge may occur during the offset of an inhibitory sensory response (rebound burst), or during the sudden excitation of thalamic neurons during a period of quiescence. Therefore, the presence of an excitatory stimulus during the lack of background tonic firing will amplify the novel stimulus. In terms of initial signal detection, the more difficult a stimulus is to detect (i.e. less contrast to surroundings), the greater the detection advantage of burst mode over tonic mode (Guido et al., 1995; Sherman, 2001a).

In burst mode, dLGN neurons respond more selectively to sudden changes in the visual world, and will not respond well to static images or gradual changes; in this way, burst mode is more effective during a visual search, when less accurate analysis is sufficient for target acquisition. This is especially useful when attention is directed elsewhere; a burst discharge can activate the cortex for novel, interesting, or potentially

dangerous stimuli. After initial detection by burst mode, the cell can switch to tonic mode for more accurate stimulus analysis (Sherman, 2001b).

In regards to cortical activation, conductances activated by a burst discharge in relay neurons will transmit through a large majority of synapses, to produce a maximal excitatory response in cortical cells (Swadlow and Gusev, 2001). Contrastingly, tonic mode only produces successful transmission in a fraction of active synapses, so this will result in smaller excitatory currents at the cortex. These findings further reiterate the efficiency of burst mode in activating the cortex.

B3. Searchlight and wake up call hypotheses

From the experimental observations discussed above, two related hypotheses have been attributed to the role of burst firing, which are the searchlight and wake up call hypotheses.

The *searchlight hypothesis* was coined by Crick (1984), who first recognized the role of the thalamus in intensifying novel and salient information transfer to the cortex. He hypothesized that a "searchlight" should be able to sample activity in the cortex and thalamus, and decide where the action originates. It should then be able to intensify thalamic output to the respective region of the cortex, and then move on to the next region that is demanding attention. The TRN is especially pertinent in this hypothesis, since its inhibitory neurons can effectively select a subset of neurons that correspond to the activated regions, and inhibit neighboring, or "noisy" regions of the relay nucleus. The TRN's ability to regulate the membrane potential of thalamic neurons by providing inhibition, will promote burst mode, and send salient information to the cortex more readily (Crick, 1984a).

The wake up call hypothesis, is a different interpretation for the function of burst discharge in activating the cortex. This hypothesis states that burst mode maximizes initial stimulus detection, and sends a "wake up call" to the cortex, alerting it that something has changed in the environment. A sudden novel stimulus in a previously unattended region of visual space could elicit a burst discharge in the relay neuron, which would be more effective in initial stimulus detection, and initiate a crude analysis of the object. Once the change is detected by the cortex, the relay neuron can switch to tonic mode so that the new object can be analyzed more effectively (Sherman, 2001a).

C. Other membrane conductances in relay neurons

Aside from I_T, there are several membrane conductances, which are responsible for various firing properties of thalamic neurons.

I_{Na} is the traditional voltage-dependent Na^+ current, responsible for action potential generation and firing once membrane potential exceeds threshold. Briefly, Na^+ channels can be in any of the three different states: deinactivated, activated, or inactivated, based on membrane voltage. At resting membrane potential, Na^+ channels are closed and deinactivated; following a suprathreshold membrane depolarization (past -50 mV), there will be an increase in I_{Na}, allowing an influx of ions. This further depolarization due to Na^+ influx will inactivate the channel, causing it to close, and preventing channel opening. Following a period of brief hyperpolarization from K^+ conductances, the Na^+ channel will be become deinactivated and will be able to respond to a suprathreshold input again.

Several K^+ channels contribute to the different thalamic conductances. Delayed rectifier K^+ channels are responsible for membrane repolarization following an action potential. They generally have slower kinetics than Na^+ channels, and exist in either

activated or deactivated states. These K^+ channels become activated with membrane depolarization, but due to their slower kinetics, they open after Na^+ channels become deinactivated. Following K^+ channel activation, K^+ flows out of these channels to hyperpolarize the membrane.

The transient K^+ (I_A) current is another voltage-gated channel that is responsible for fast action potential dynamics. I_A is similar to other K^+ currents, but it has a lower activation threshold, and rapid kinetics for activation and inactivation. It is different from the previously described K^+ channel in that it has three states: activated, inactivated, and deinactivated. I_A activation causes hyperpolarization of the membrane, acting to offset membrane depolarization. In tonic mode, I_A acts to delay and reduce the frequency of action potentials, thereby delaying action potential saturation from occurring at high firing frequencies.

I_{KCa} is a type of Ca^{2+}-dependent K^+ conductance, which combines with Ca^{2+} influx following activation of I_T, to produce a large hyperpolarization, contributing to rhythmic bursting. Following I_T inactivation, I_{KCa} allows the cell to repolarize so that another LTS can be generated.

There are several high-threshold Ca^{2+} conductances in thalamic neurons. N- and L-, type Ca^{2+} channels, among others, are prevalently expressed and are located in the dendrites and synaptic terminals. L-type Ca^{2+} channels produce a long lasting current, and slow inactivation. N-type Ca^{2+} channels activate more rapidly than L-type Ca^{2+} channels, and the threshold of activation is ~-20mV. Since these channels are activated during action potential innervation, they facilitate Ca^{2+} entry into the terminal, and are the link between action potential innervation and neurotransmitter release. These channels are especially

important in dendritic integration, since they ensure that distal dendritic inputs are strong enough to influence the soma and axon. Several different Ca^{2+}-dependent K^+ conductances are activated following activation of high-threshold Ca^{2+} channels, as well. They are fast to activate and help repolarize the cell and produce the after-hyperpolarization phase of the action potential.

I_h, is the hyperpolarization-activated cation current, and is also known as the "pacemaker potential." Activation of this current in response to membrane hyperpolarization (-70 to -85mV) causes an influx of mixed cations, leading to a net inward current (McCormick and Pape, 1990b). I_h is responsible for the "sag current" seen at hyperpolarized potentials, and has a slow activation time constant (200 ms). I_h can be activated following I_T deinactivation and membrane hyperpolarization. The slow depolarization induced by I_h can activate I_T again, leading to the propagation of oscillatory bursting.

There are Na^+, K^+, and Cl^- leak currents, which are voltage-independent, and contribute to the resting membrane potential of relay neurons, which can be between -65 to -75 mV. There is also a persistent Na^+ conductance that is activated by strong depolarization and is non-inactivating. When activated, these channels are responsible for sustained, tonic firing (Jahnsen and Llinas, 1984a, c).

D. Oscillatory rhythmic bursting

The precise interplay of the conductances discussed above, contributes to the firing mode of the thalamic neuron and how it responds to incoming inputs. A unique property of thalamic neurons is the ability to burst in a repetitive or oscillatory manner. Rhythmic, or

more synchronized, burst discharge is the pattern that is characteristic of slow-wave sleep or absence epilepsy.

Rhythmic bursting is a form of oscillatory activity, and it involves synchrony among relay neurons. Three types of rhythmic bursting include intrinsic rhythmic bursting, spindle waves, and absence seizures. ***Intrinsic rhythmic bursting***, is the only intrinsic form of oscillatory activity in relay neurons, and occurs at a frequency of 0.5-4 Hz (McCormick and Prince, 1988; McCormick and Pape, 1990b; Leresche et al., 1991; Dossi et al., 1992). Each burst in slow rhythmic bursting is generated by the activation of I_T. The resultant burst discharge is then followed by activation of various K^+ conductances, which leads to membrane repolarization. Between each burst, I_h is activated by membrane hyperpolarization. The slow depolarization due to I_h activates I_T and is responsible for initiating another LTS. Since I_h is the only non-leak conductance that is active between bursts, its effects dominate the membrane potential, which can lead to perpetuating the cycle of bursting.

Burst firing patterns are modulated by I_h; decreasing I_h results in a decrease in the frequency of bursting, and increase in the amplitude of each LTS. Increasing I_h causes an increase in the frequency of bursting, and a decrease in the amplitude (McCormick and Pape, 1990b). Together, the rate of bursting is determined by the amplitude and kinetics of $I_{h/T,}$ and the amplitude of resting leak conductances. During this cycle of oscillations, if the cell undergoes membrane depolarization, it will cause a switch to tonic firing due to I_T inactivation, coupled with the lack of I_h activation (Jahnsen and Llinas, 1984b; Bal and McCormick, 1993; Huguenard and Prince, 1994; Bal et al., 1995).

Intrinsic rhythmic bursting can be independently generated by relay neurons. Steriade et al. (1985) showed that slow rhythmic bursting is still evident following cortical deafferentation, demonstrating how this form of bursting is does not require cortical influence (Steriade et al., 1985).

The other types of rhythmic bursting, known as spindle waves and absence seizures, occur through the interaction with other brain regions, and are not generated intrinsically by relay neurons. ***Spindle waves*** occur via interactions with the TRN, and are present exclusively during slow-wave sleep (Steriade and Deschenes, 1984). The firing rate of spindle waves occurs at a frequency between 7-14 Hz (Steriade et al., 1993). Spindle waves have been shown to originate in the thalamus and are projected to the cortex (observed in electroencephalogram recordings). Spindle initiation occurs when an incoming stimulus coincides with a shift in the voltage dependence of I_h, due to reduced intracellular Ca^{2+} concentration. The stimulus causes a burst in the relay neuron, which excites TRN neurons; TRN excitation produces feedback inhibition to relay neurons, initiating rebound bursting. This cycle of spindle oscillations continues until the voltage dependence of I_h shifts with increased intracellular Ca^{2+} concentrations (Steriade and Deschenes, 1984; Pare et al., 1987; von Krosigk et al., 1993).

The thalamic origin of initiation of spindle waves is demonstrated by a series of experiments, where the removal of the cortex maintains thalamic oscillations, but the removal of the thalamus prevents formation of oscillations in the cortex (Morison and Bassett, 1945; Villablanca, 1974). Other experiments have gone more in depth into the thalamic origin of spindles, showing the necessity of the TRN in generating spindle oscillations (Steriade et al., 1985; Contreras et al., 1993; Bal et al., 1995). These network

oscillations are generated by the interaction between relay neurons and the TRN, as shown by a series of experiments by Steriade and Deschenes (1984). When the synaptic connection between TRN and relay neurons is blocked by TTX, oscillations in the intrinsic rhythmic bursting range resulted (0.5-4 Hz) (Steriade and Deschenes, 1984). So it was concluded that spindle waves occur as a result of alignment of similar rhythms in connecting TRN and relay neurons, to produce the 7-14 Hz frequency of oscillations (Leresche et al., 1991; Soltesz et al., 1991; McCormick, 1992). The observation that thalamic relay neurons and TRN neurons displaying synchrony during generation of spindle waves has been mainly attributed to local axonal, dendrodendritic, and electrical connections between TRN neurons (Deschenes et al., 1985; Steriade and Llinas, 1988; Steriade et al., 1990c; Landisman et al., 2002). Large scale synchrony between TRN neurons can then exert a coordinated response onto relay neurons (Contreras and Steriade, 1996).

The last type of rhythmic oscillation are ***absence seizures***, which are a paroxysmal condition, where dysfunction occurs in the thalamocortical loop, to produce epileptic seizures (Steriade and Deschenes, 1984; Gloor and Fariello, 1988; Steriade and Llinas, 1988). Spike-wave complexes that occur during absence seizures occur at a frequency of 3-4 Hz, which is associated with a loss of consciousness, as seen in the clinical condition. Unlike spindle waves, spike-wave seizures originate in the intracortical synaptic network. During spike-wave seizures, TRN neurons are strongly activated by corticothalamic projections, producing prolonged inhibitory activity on relay neurons, which can subsequently generate and sustain oscillatory firing (Meeren et al., 2002; Steriade and Amzica, 2003). The strong inhibition coupled with increased membrane conductance in relay neurons could be a mechanism for the failed relay of incoming sensory inputs,

manifesting in lapses of unconsciousness in absence epilepsy (Steriade, 2005). It has been shown that the activation of $GABA_B$ receptors in relay neurons leads to a larger rebound burst, which can then lead to the further excitation of more TRN neurons, perpetuating the seizures (Liu et al., 1992). These findings have led to the use of $GABA_B$ antagonists to decrease absence seizures, along with other agents that can reduce LTS amplitude and decrease aberrant spike-wave activity .

D1. Behavioral implications of rhythmic activity

Burst and tonic firing modes significantly influence how thalamic neurons respond to sensory inputs. In dLGN relay neurons, rhythmic burst discharge during slow-wave sleep or drowsiness is associated with reduced responsiveness to incoming sensory information (Coenen and Vendrik, 1972; Livingstone and Hubel, 1981; Steriade et al., 1990b). These findings led to the assumption that burst firing represents a functional disconnection of the relay cell from driving inputs. It has been hypothesized that although the brain may not be actively transmitting sensory inputs during the sleeping state, the function of rhythmic oscillations may be to maintain the forebrain in a state of readiness for a quick transition to the aroused state if a threatening stimulus presents itself (Steriade and Llinas, 1989). In contrast, the awake state is associated with the absence of rhythmic bursting, since neurons are more depolarized during arousal (Hirsch et al., 1983; McCarley et al., 1983), which promotes tonic firing.

Oscillatory bursting is associated with the generation of rhythms, which do not reflect the pattern of sensory inputs, so it may contribute to the reduction of sensory perception during periods of sleep or drowsiness (Coenen and Vendrik, 1972; Livingstone and Hubel, 1981; Steriade et al., 1993). Rhythmic oscillations lead to reduced sensory

information transfer through several possible mechanisms: 1) Since burst discharge occurs at a frequency of <15 Hz, due to the time requirements for inactivation of I_T between bursts, thalamic neurons in burst mode can only respond to excitatory inputs that arrive at low frequencies 2) Since rhythmic bursting displays endogenous rhythmic patterns from interactions with other oscillators (relay neurons, TRN), these influences will interfere with linear transmission of sensory information 3) Since the LTS is initially generated by a large, slow inhibitory current, it requires a greater threshold for EPSP generation, which can distort sensory information transfer (Steriade and Deschenes, 1984; McCormick and Feeser, 1990; McCormick, 1992). However, it is important to note that while burst mode shows reduced sensory information transfer, this reduction is not due to altered retinal sensory inputs (Mukhametov and Rizzolatti, 1970), signifying the disruption is occurring at thalamic levels.

1.4 Other Inputs/Outputs involved in thalamic modulation

While the firing mode of thalamocortical neurons can be shifted based on the conductances discussed above, there are several modulators that are released from other brain regions, which can also affect the membrane potential of thalamic neurons and alter firing mode.

A. Glutamatergic inputs from sensory and corticothalamic afferents

Relay neurons primarily receive glutamatergic inputs from primary sensory afferents and corticothalamic afferents (Baughman and Gilbert, 1980; Crunelli et al., 1987a{Kemp, 1982 #1668; Deschenes and Hu, 1990; Scharfman et al., 1990). The sensory and corticothalamic synapses activate different glutamatergic receptors, which can influence thalamic firing mode.

A1. Sensory afferents

Sensory afferents typically activate ionotropic glutamate receptors (AMPA and NMDA receptors), to initiate quick, transient responses to incoming stimuli (Mayer and Westbrook, 1987; Scharfman et al., 1990; Sillito et al., 1990a; Sillito et al., 1990b; Jones and Sillito, 1992). Briefly, ionotropic receptors mediate fast excitatory currents, with response duration of ~10 ms. Activation of ionotropic receptors produces direct ion channel opening and cation influx. AMPARs are permeable to Na^+ and K^+ primarily. NMDARs are unique, in that they have a voltage dependency due to the Mg^{2+} blockade of the channel pore (Nowak et al., 1984). NMDARs are activated at depolarized membrane potentials (which will effectively remove the Mg^{2+} block from the channel pore), and display a longer response (tens of ms) than AMPAR-dependent responses. Along with mixed Na^+ and K^+ influx, NMDARs are also permeable to Ca^{2+}, which can activate downstream second messenger pathways.

AMPA and NMDA receptors may be differentially activated based on the nature of incoming sensory information. In the VB, it has been shown that while ionotropic glutamate receptors are activated to mediate transmission of incoming somatosensory stimuli, the receptor subtypes are differentially recruited based on duration of the stimulus. For example, NMDARs are preferentially responsible for transmitting longer duration (> 1s) somatosensory activity, while AMPARs are primarily activated for shorter duration activity (Salt and Eaton, 1989). Primary sensory afferents do not play a major role in changing relay neuron firing mode, since the fast, transient effects, are too rapid to inactivate I_T.

A2. Corticothalamic afferents

Corticothalamic afferents can activate both ionotropic and metabotropic glutamate receptors (mGluRs) (McCormick and von Krosigk, 1992). Group 1 (types 1 and 5) and group 2 (types 2 and 3) mGluRs are expressed in the thalamus (Masu et al., 1991). mGluRs do not directly open ion channels, but activate downstream second messenger pathways, which ultimately lead to altered K^+ conductance. mGluRs have a slow time to depolarization (>10 ms latency), due to second messenger activation, and produce a prolonged effect (lasting hundreds of milliseconds to several seconds in duration) (McCormick and von Krosigk, 1992). mGluRs are activated by high frequency trains of afferent stimulation, since the receptors are located farther from the synaptic junction. mGluR-mediated excitation is resultant from the downstream reduction in the K^+ current, leading to membrane depolarization.

Corticothalamic afferent activation can produce both fast EPSPs, mediated by NMDA and non-NMDARs (Hull, 1968; Kalil and Chase, 1970; Ahlsen et al., 1982; Lindstrom and Wrobel, 1990; Scharfman et al., 1990), and slower EPSPs, mediated by mGluR activation (McCormick and von Krosigk, 1992). Since mGluR activation produces a prolonged depolarization, it is particularly effective in shifting the firing mode of thalamic neurons from burst to tonic mode (Deschenes and Hu, 1990; Godwin et al., 1996). mGluR activation is too slow to activate I_T directly, due to T-type Ca^{2+} channel kinetics, but mGluRs cause the prolonged depolarization necessary to inactivate I_T (Gutierrez et al., 2001). In this way, mGluRs have the ability to inactivate I_T without directly activating it; this will cause the cell to switch to tonic mode, while simultaneously reducing burst activity (Sherman and Guillery, 2006). Therefore, corticothalamic feedback is more effective in mediating the shift

in firing mode and robustly modulating the activity of thalamic relay neurons, through activation of mGluRs.

B. GABAergic inputs from TRN and interneurons

Thalamic relay neurons also receive GABAergic projections from TRN neurons, and interneurons (Sterling and Davis, 1980; Hendrickson et al., 1983; Ohara and Lieberman, 1985; Montero and Zempel, 1986). TRN neurons and interneurons can activate $GABA_A$ and $GABA_B$ responses on relay neurons, which make up the ionotropic and metabotropic receptors, respectively (Crunelli et al., 1988a; Cox et al., 1997). $GABA_A$ receptor activation causes direct chloride channel opening, leading to fast membrane hyperpolarization. $GABA_B$ receptor activation induces a slow increase in K^+ channel conductance, resulting in a longer lasting effect (hundreds to thousands of milliseconds in duration), which causes a strong hyperpolarization of the membrane (Hirsch and Burnod, 1987; Crunelli et al., 1988b; Thomson, 1988). Similar to mGluR activation, $GABA_B$ activation can alter the firing mode of relay neurons; the slow effects of $GABA_B$ activation on the thalamocortical neuron can produce a strong hyperpolarization that can deinactivate I_T, which, in turn, can lead to burst discharge.

The firing mode of TRN neurons can impact the amount of inhibition it imparts on relay neurons. Burst firing in TRN neurons has been shown to activate $GABA_A$ and $GABA_B$ receptors on relay neurons, while tonic discharge in the TRN activates $GABA_A$ receptors (Kim et al., 1997). Since the TRN can activate both $GABA_{A/B}$ receptors, it has a major role in switching the firing mode of relay neurons, as well as providing inhibition for focused sensory transmission.

C. Impact of other afferents

C1. Brainstem afferents

PBR afferents play a role in mediating arousal and activation of relay neurons during REM sleep (Steriade et al., 1990a). PBR afferents are primarily cholinergic, and activate both ionotropic nicotinic acetylcholine receptors, and metabotropic, muscarinic (M1) AChRs (Curro Dossi et al., 1991). Nicotinic receptor activation leads to mixed cation influx, and produces fast excitatory potentials. Muscarinic receptor activation leads to the reduction of K^+ leak currents, thereby slowly depolarizing the membrane, which can shift the neuron into tonic firing mode (McCormick, 1991; Lu et al., 1993).

It has been shown that some cholinergic terminals also contain nitric oxide (NO) synthase (Bickford et al., 1993). NO can depolarize the membrane and cause relay neurons to switch from burst to tonic firing, so it has been hypothesized that NO release may complement cholinergic effects (Pape and Mager, 1992). NO has also been shown to modulate retinal afferent activity (Cudeiro et al., 1996), which may be a mechanism by which brainstem afferents can modulate sensory information transfer.

The PBR also contains some noradrenergic projections, which activate metabotropic noradrenergic receptors on relay neurons, α_1 and β adrenoceptors. α_1 adrenoceptors increase the excitability of relay neurons by reducing K^+ leak conductance, and β adrenoceptors change the voltage dependency of I_h, which can thereby depolarize the membrane and also switch the firing mode (McCormick and Prince, 1988). The increase in I_h can promote oscillatory burst firing (Pape and McCormick, 1989).

C2. Dorsal Raphe Nucleus Afferents

The dorsal raphe nucleus produces serotonergic afferents to thalamic neurons, which can influence the sleep wake cycle. The effects of serotonin on relay neurons has not been consistent; *in vivo* experiments have shown that serotonin can inhibit relay neuron activity (Curtis and Davis, 1962; Yoshida et al., 1984; Kayama et al., 1989), but *in vitro* slice experiments show that serotonin can depolarize relay neurons by modulating I_h (McCormick and Pape, 1990a). More recent *in vitro* studies have shown that serotonin can activate both a direct hyperpolarization of relay neurons through an increase in K^+ conductance, and also an indirect inhibition through the activation of local interneurons (Monckton and McCormick, 2002).

C3. Tuberomammillary nucleus afferents

The tuberomammillary nucleus of the hypothalamus sends histaminergic afferents to the thalamus, and is thought to be involved in arousal. Interestingly, few histaminergic synapses have been seen using electromicrograph imaging (Uhlrich et al., 1993), suggesting that these afferents might release histamine in a different manner (e.g. release histamine into extracellular spaces rather than forming synaptic junctions). Histamine acts through H1 metabotropic receptors to reduce K^+ conductance, and promote tonic firing (McCormick and Williamson, 1991).

C4. Inputs to the TRN

The TRN has the same inputs as listed above for relay nuclei, but the TRN also receives GABAergic inputs from the basal forebrain. The TRN receives local TRN-TRN connections (axodendritic, dendrodendritic, gap junctions), as well as thalamocortical and corticothalamic afferents, since these projections both send collaterals through the TRN

(Scheibel and Scheibel, 1966; Deschenes et al., 1985; Ohara and Lieberman, 1985; Cox et al., 1997; Pinault et al., 1997; Landisman et al., 2002).

1.5 Synaptic properties

Now that the different inputs to the thalamus have been discussed, it is important to note specific synaptic properties that impact how information is transferred to relay neurons. The ways in which synapses respond to paired-pulse stimulation can yield insight into the synaptic and vesicular properties of the afferents, which can have a significant impact on thalamic synaptic plasticity. Additionally, it will be imperative to understand the relationship between postsynaptic firing mode and synaptic features, since these factors can contribute to synaptic plasticity.

A. Paired-pulse properties

How synapses respond to presynaptic activation can be investigated by the paired-pulse experimental paradigm, where two stimulation pulses are given in close proximity, and the size of the two synaptic responses can be compared. Paired-pulse experiments can yield information about the synapse and the afferent from which it originates.

Corticothalamic afferents display *paired-pulse facilitation* (interstimulus interval: 1-20 Hz). Comparing the size of the EPSCs following each stimulation shows that the second EPSC is larger than the first (**Fig. 1.5A**). Contrastingly, retinogeniculate (or other primary sensory) afferents display *paired-pulse depression,* where the first EPSC is larger than the second. (**Figure 1.5B**) (Thomson, 2000). The mechanism contributing to these different outcomes is dependent on several factors, which will be discussed in the next section. This experimental method can be a useful tool for identifying the afferent that is being stimulated, and to determine specific synaptic properties.

The mechanism of action that is manifested as paired-pulse facilitation or depression is resultant from either pre- or postsynaptic effects. The ***presynaptic theory*** states that the change in the amplitude of the second response is a result of enhanced presynaptic neurotransmitter release. Different afferents display variable probabilities of release, depending on synaptic vesicles and intracellular ion concentrations. A larger probability of release (high probability of release synapses) will lead to more synapses releasing neurotransmitter and this will produce a larger first EPSP, and paired-pulse depression will be observed at these synapses. A smaller probability of release (low probability of release synapses) will produce a smaller first EPSP, and paired-pulse facilitation will be observed at these synapses. The probability of release is intrinsically determined by the size of the readily releasable pool of synaptic vesicles (i.e. number of vesicles, or size of individual vesicles) at the axon terminal, and/or the magnitude of residual Ca^{2+} at the axon terminal. High Ca^{2+} concentrations at the terminal following the first stimulus will cause greater neurotransmitter release with a subsequent stimulus, observed in paired-pulse facilitation (Lando and Zucker, 1994; Dunlap et al., 1995; Matthews, 1996; Reuter, 1996). A ***postsynaptic theory*** of paired-pulse observations assumes that the probability of neurotransmitter release is unchanged, and paired-pulse effects are related to the temporal summation of the EPSP and activation of postsynaptic NMDARs. Paired EPSPs can be sufficient to depolarize the postsynaptic cell and relieve the Mg^{2+} block of the NMDAR pore. Subsequent action potentials will then increase the chances of the postsynaptic cell being able to generate an EPSP, since it is already activated. The result will be paired-pulse facilitation. The pre- and postsynaptic contributions to paired-

pulse effect are differentially evident at various synapses in the central nervous system, and can be used to investigate features of synaptic plasticity.

B. Dynamics of high/low probability of release synapses

The paired-pulse dynamics of thalamocortical synapses can also impact how information is transferred to the cortex, depending on the firing mode of the relay neuron. Thalamocortical synapses display a high probability of release, and as discussed previously, the thalamic firing mode can dramatically change how information is transferred to the cortex. In tonic mode, a relay neuron will generate an EPSP in the cortex with each action potential. However, if the neuron is in burst mode, only the first action potential will produce a strong postsynaptic response, since the EPSPs will display depression with successive spikes (Sherman, 2001a). In this way, strong cortical activation by burst mode can be used to initially stimulate the cortex, but further information processing may require the transition to tonic firing mode.

1.6 Synaptic Plasticity

Since we know that thalamic firing mode can impact information transfer, one aspect of this study is to investigate synaptic plasticity within the thalamocortical circuit, and determine how the firing mode can influence information transfer to the cortex. This study will primarily examine the effects of synaptic plasticity at corticothalamic synapses and the implications that plasticity may have in thalamocortical communication. To further understand the mechanism of synaptic plasticity at thalamocortical synapses, an overview of synaptic plasticity will be reviewed. The general mechanisms of long-term synaptic plasticity in the well-studied synapses of the hippocampus will form a basis for the experimental manipulations tested in the subsequent chapters.

A. Hippocampal long-term synaptic plasticity

Synaptic plasticity has been well documented at different hippocampal regions in relation to memory formation. In 1949, Hebb formed a pivotal postulate about memory storage in the brain. He stated that, " When an axon of cell A is near enough to excite cell B, and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (Hebb, 1949). From this early postulate, Hebb's theory continues to hold true in many neural synapses, in that coincident neural activity of two connected cells have the ability to alter the strength of that synapse.

Synaptic plasticity can be presynaptic, causing enhanced neurotransmitter release; postsynaptic, causing enhanced synaptic currents in response to unchanging neurotransmitter release; or both of these processes can contribute to plasticity (Bliss and Lomo, 1973). In the seminal study by Bliss and Lomo (1973), the researchers induced long-term potentiation (LTP) in the dentate area of the hippocampus by stimulating the perforant pathway. They showed that by evoking a high-frequency train of stimulation to the perforant pathway, synaptic transmission was increased for greater than six hours. Since those initial studies, LTP has now been demonstrated in several brain regions, and can be expressed through many different neural mechanisms.

This discussion will include the mechanisms involved in long-term plasticity at the well-studied glutamatergic synapses of the hippocampus, the CA3-CA1 synapses and the mossy fiber synapses. The mechanisms contributing to plasticity at these synapses have been shown in various other brain regions, and can be recalled when investigating plasticity at synapses of the thalamocortical circuit.

A1. NMDAR-dependent synaptic plasticity (CA3-CA1)

LTP at the CA3-to-CA1 synapse of the Schaffer collateral pathway in the hippocampus results in a form of LTP requiring postsynaptic activation of NMDARs, referred to as NMDAR-dependent LTP (Collingridge and Bliss, 1987; Malenka et al 1989). High-frequency stimulation causes glutamate release, which binds to both postsynaptic NMDA and non-NMDA receptors. NMDARs are referred to as *coincidence detectors*, since they can detect simultaneous pre- and postsynaptic activity. As discussed in Section **1.4**, NMDARs require both ligand binding (from presynaptic terminal) and voltage-dependency (postsynaptic membrane potential) to become activated. In this way, NMDARs are an ideal candidate to associate the activity of pre- and postsynaptic communication. At the CA3-CA1 synapse, NMDAR activation, and postsynaptic Ca^{2+} influx via NMDARs are both required to produce LTP (Collingridge et al., 1983; Kauer et al., 1988; Malenka et al., 1988; Nicoll et al., 1988; Collingridge and Bliss, 1995).

The postsynaptic depolarization required to activate NMDARs can be induced experimentally by repetitive tetanic stimulation of the synapses, or by administering a *pairing protocol*, by depolarizing the postsynaptic cell, while synaptically stimulating the presynaptic afferent (Nicoll and Malenka, 1999). Following NMDAR activation, postsynaptic Ca^{2+} influx can then signal an enzymatic cascade, which can activate various other pathways, leading to structural changes, altered channel conductance, and protein synthesis (discussed in detail below) (Bliss and Collingridge, 1993). These processes have been shown to contribute to the postsynaptic expression of LTP at these synapses.

A2. Presynaptic LTP: Mossy fiber-CA3 synapses

Hippocampal mossy fiber-to-CA3 LTP is another form of long-term plasticity, however, this type is NMDAR-independent and has a presynaptic origin (Harris and Cotman, 1986; Xiang et al., 1994; Maeda et al., 1997; Nicoll and Schmitz, 2005). Increased presynaptic Ca^{2+} levels leads to the activation of Ca^{2+}/calmodulin-activated adenylyl cyclases (Xia et al., 1993). Adenylyl cyclase activation, which leads to a rise in presynaptic cAMP levels are both necessary to induce mossy fiber LTP. Downstream of cAMP production, subsequent PKA activation contributes to the enhanced, sustained neurotransmitter release (Huang et al., 1994; Weisskopf et al., 1994; Huang et al., 1995; Lopez-Garcia et al., 1996).

A3. Presynaptic LTD: Mossy fiber-pyramidal cell synapses

While presynaptic mossy fibers demonstrate LTP at mossy fiber-CA3 synapses, they also display long-term depression (LTD) at mossy fiber-pyramidal cell synapses. LTD is a lasting suppression in synaptic transmission, and can be induced with lower frequency stimulation of afferents (~1 Hz) (Kobayashi et al., 1996). LTD at mossy fiber synapses is also presynaptic and occurs due to the activation of presynaptic metabotropic glutamate receptors (mGluR2) (Yokoi et al., 1996), and a rise in presynaptic Ca^{2+} following stimulation (Yokoi et al., 1996; Tzounopoulos et al., 1998). Increased Ca^{2+} levels then lead to the activation of other enzymatic processes. Mossy fiber LTD occurs as a result of reduced adenylyl cyclase activity, leading to a downstream decrease in PKA activity (Tzounopoulos et al., 1998).

B. General mechanisms of plasticity

Now that a brief overview has been given regarding some examples of synaptic plasticity in the hippocampus, the common, underlying mechanisms that produce synaptic plasticity in different brain regions will be discussed.

B1. Ca^{2+}-dependent processes

In most forms of synaptic plasticity, Ca^{2+} is necessary to induce alterations in synaptic efficacy. In presynaptic LTP, neurotransmitter release is mediated by Ca^{2+} influx through channels located at the axon terminal. In NMDAR-dependent LTP, following initial postsynaptic Ca^{2+} entry, the association of Ca^{2+} to calmodulin leads to the activation of numerous downstream kinases and phosphatases (i.e. CaMKII, PKC, calcineurin) directly, as well as indirectly activating others (PKA, PP1) (Lee et al., 2000). Kinase activation can induce release of Ca^{2+} from intracellular stores, also leading to further increases in postsynaptic concentrations. The balance between kinase and phosphatase activity depends on the concentration and temporal profile of Ca^{2+} transients (Lisman, 1985). The stimulation paradigm can affect the magnitude of Ca^{2+} transients, which will impact how plasticity is manifested. In general, high-frequency stimulation induces LTP, since it produces a brief and larger Ca^{2+} transient. In contrast, low-frequency stimulation induces LTD, since it produces lower and prolonged Ca^{2+} levels (Lisman, 1985) (For review, see: (Bliss and Cooke, 2011))(Heynen et al., 2000; Karmarkar and Buonomano, 2002; Shouval et al., 2002a; Shouval et al., 2002b). An increase in Ca^{2+} that does not reach threshold for LTP can lead to the generation of short-term plasticity, which occurs over the course of 5 to 20 minutes, or LTD (Malenka and Nicoll 1999). In the traditional CA3-CA1 synapse, plasticity

is resultant from the magnitude of postsynaptic Ca^{2+} transients through NMDARs that are elicited following coincident pre- and postsynaptic stimulation (Malenka and Nicoll, 1993). In presynaptic forms of long-term plasticity, the magnitude of residual Ca^{2+} at the axon terminal can impact the timing and form of plasticity.

There are several significant signal transduction molecules that are activated downstream of Ca^{2+} entry. Many experimental studies of LTP show the activation of α-calcium-calmodulin– dependent protein kinase II (CaMKII) as a necessary step to induce LTP (Lisman, 1994; Lisman et al., 1997). CaMKII can contribute to LTP through several downstream mechanisms, such as increasing AMPAR conductance (Barria et al 1997), autophosphorylating itself, which can produce sustained effects (Crick, 1984b), and by activating other downstream enzymes.

B2. AMPA/NMDA receptors

AMPA and NMDA receptors are often co-localized at postsynaptic synapses, and receptor expression can influence long-term plasticity. In LTP expression at the CA3-CA1 synapses, the AMPAR-mediated EPSC is preferentially increased more than the NDMAR-mediated EPSC (Kauer et al., 1988; Muller et al., 1988; Asztely et al., 1992; Perkel and Nicoll, 1993). Studies have shown that this is due to postsynaptic modifications, which lead to increased AMPAR insertion or conductance through these receptors. Additionally *silent synapses* have been implicated in synaptic plasticity, which shows the differential regulation of AMPA and NMDA receptors following stimulation. Prior to stimulation, synapses are considered "silent" or not functional, if they contain few, if any AMPARs (although NMDARs may be present). AMPAR insertion following stimulation can the make that synapse functional, by causing AMPAR and NMDAR activation (Isaac et al., 1999).

Therefore, differential expression of AMPA and NMDA receptors has been examined in various plasticity experiments, when determining the locus of expression for plasticity.

B3. Retrograde messengers

Retrograde messengers have also been implicated in contributing to LTP at other synapses, typically when LTP originates postsynaptically. Retrograde messengers are released from the postsynaptic cell to modify presynaptic function. Examples of retrograde messengers include nitric oxide (NO), carbon monoxide, arachidonic acid, and endocannabinoids, among others. NO release, for example, can retrogradely activate presynaptic signaling cascades, which have been shown to increase neurotransmitter release at certain synapses (Williams et al., 1993; Kato and Zorumski, 1996; Hawkins et al., 1998).

B4. Structural alterations

Structural changes in size and shape of the pre- or postsynaptic region can contribute to changes in synaptic efficacy. There can be an increase in the size or number of active zones at either pre- or postsynaptic regions. Postsynaptic spines have been shown to change volume depending on LTP or LTD induction, as a result of altered transcription and synthesis of proteins in the spine (Harris et al., 2003).

B5. Altered neurotransmitter release

Presynaptic long-term plasticity is often a result of enhanced neurotransmitter release, as is observed in mossy fiber LTP (Tsien and Malinow, 1990). Presynaptic release can be altered by several factors, such as kinase activation, as discussed above. Additionally, ion channels can be implicated in altered presynaptic release, by changing the excitability of the terminal. For a review, see Meir et al. (1999). Increased Ca^{2+} channel

conductance can lead to enhanced neurotransmitter release, mediated through voltage-dependent Ca^{2+} channels. Additionally, blockade of K^+ channels have been shown to replicate LTP in CA1 neurons.

C. Spike-timing dependent plasticity and action potential backpropagation

The previous section has shown the impact of Ca^{2+} transients in inducing plasticity. Backpropagating action potentials are another Ca^{2+}-dependent mechanism by which enhanced dendritic Ca^{2+} transients can be produced in postsynaptic cells. Action potentials normally propagate from the axon initial segment down towards the terminal, but in many cells, action potentials can simultaneously propagate backwards as well, to infiltrate the dendrites. (Gasparini and Migliore, 2014). This occurs through active conduction through the dendrites via voltage-dependent ion channels (Stuart and Sakmann, 1994; Spruston et al., 1995; Helmchen et al., 1996; Johnston et al., 1996). The to degree to which backpropagating action potentials will innervate the dendrites is, however, dependent on the density of voltage-dependent channels, neuronal activity and morphology (Migliore and Shepherd, 2002; Magee, 2008; Gasparini and Migliore, 2014).

In thalamocortical neurons specifically, dual somatic and dendritic recordings have shown that somatic action potentials backpropagate into the dendrites. The distance of backpropagation was affected by the extensive dendritic branching of relay neurons. Relay neurons were shown to contain non-uniform distributions of Na^+ and Ca^{2+} channels, but a fairly uniform distribution of K^+ channels along the dendrites (Williams and Stuart, 2000). Since backpropagation of action potentials is yet another mechanism to increase dendritic Ca^{2+} transients, action potential backpropagation could contribute to increased dendritic Ca^{2+} in the postsynaptic neuron, without requiring NMDAR activation.

Backpropagation of action potentials could also be a mechanism to facilitate synaptic integration at dendritic locations (Markram et al., 1997). By producing a large Ca^{2+} transient at distal dendritic locations, incoming synaptic inputs will be more likely to be propagated to the soma. In relay neurons, this feature could be especially relevant, since corticothalamic inputs primarily form synapses at distal locations, where relay neurons express active conductances (Christie et al., 1995; Williams and Stuart, 2000; Crandall et al., 2010; Errington et al., 2010). Together, these features could be critical in enhancing synaptic integration and inducing changes in synaptic efficacy following coincident pre- and postsynaptic stimulation.

Spike-timing-dependent plasticity (STDP) is a mechanism that integrates the concept of backpropagating action potential and coincident synaptic activity to produce plasticity. STDP depends on the timing of both presynaptic activity and postsynaptic spiking. Timing between pre- and postsynaptic activity has been shown to be especially important in synaptic integration; for example, if one cell is systematically active before another, the firing of the first cell could have a causal link to the firing of the second cell. This link can be maintained, or enhanced, by increasing synaptic efficacy (Markram et al., 2012), which forms the basis of STDP. The most significant feature of STDP is the dependence on the temporal order of pre- and postsynaptic spike activity, which can determine the direction and extent of synaptic changes (Bi and Poo, 1998). In neocortical neurons, the coincidence of action potentials and EPSPs induces synaptic changes, depending on the timing between action potentials and the EPSP. Postsynaptic spiking following presynaptic activation typically induces LTP, while postsynaptic spiking preceding presynaptic activation caused LTD, and both effects were shown to be

dependent on NMDAR activation (Christie et al., 1996b, a; Duguid and Sjostrom, 2006; Pawlak et al., 2010; Rodriguez-Moreno et al., 2010). These findings show that STDP and timing between pre- and postsynaptic activity can lead to synaptic changes.

D. Short-term plasticity

Activity-dependent short-term plasticity can be expressed in different forms, based on the duration of action. Different classifications of short-term plasticity have been made, known as depression, facilitation, and augmentation/posttetanic potentiation (PTP). Depression and facilitation typically refer to changes lasting on the order of hundreds of milliseconds to seconds. An example would be paired-pulse facilitation and depression (discussed in Section **1.5**, **Fig. 1.5**). Augmentation occurs over a longer duration of time than facilitation, and represents an enhancement of EPSP amplitude with successive stimuli. Augmentation can last for seconds up to minutes (Magleby and Zengel, 1976; Granseth and Lindstrom, 2004). Sustained presynaptic activation at high frequencies can induce post-tetanic potentiation (PTP), which is more slowly developing than facilitation or augmentation, and also decays slowly (tens of seconds to tens of minutes). In our experiments, we observe synaptic alterations that last on a similar time scale (15-20 minutes), so we will be examining the mechanisms for post-tetanic potentiation (Thomson, 2000), which may contribute to plasticity observed at thalamocortical synapses.

D1. Postsynaptic factors

While presynaptic mechanisms for short-term plasticity are often regarded as the dominant theory for synaptic alterations, there are postsynaptic receptor mechanisms that contribute to short-term plasticity, as well. When the probability of presynaptic release is high, postsynaptic receptors can become saturated or sensitized, which can limit the extent

of plasticity at the synapse (Trussell et al., 1993; Wadiche and Jahr, 2001; Foster et al., 2002; Xu-Friedman and Regehr, 2004). Postsynaptic receptor modifications can account for some forms of short-term depression.

D2. The role of presynaptic Ca^{2+}

Short-term plasticity is often attributed to changes in presynaptic neurotransmitter release. Similar to long-term plasticity, presynaptic residual Ca^{2+} is an important factor in affecting neurotransmitter release in short-term plasticity (Burnashev and Rozov, 2005; Neher and Sakaba, 2008; de Jong and Verhage, 2009). Ca^{2+} and its association with and calmodulin and CaMKII contribute to the lasting effects of posttetanic potentiation, by leading to other downstream signaling pathways (Chapman et al., 1995; Wang and Maler, 1998; Fiumara et al., 2007).

Posttetanic potentiation has been attributed to alterations in local Ca^{2+} concentration. Tetanic stimulation could act to increase local Ca^{2+}, leading to enhanced probability of neurotransmitter release for a prolonged period of time. Saturation of Ca^{2+} buffers can also account for posttetanic potentiation, which will produce longer-lasting effects, until the buffers chelate excess Ca^{2+} (Regehr, 2012).

The increase in residual Ca^{2+} levels at the terminal activates Ca^{2+} sensors (long-lasting sensors), which will contribute to prolonged levels of Ca^{2+} that decay to baseline at a similar time course to that of posttetanic potentiation (Delaney et al., 1989; Delaney and Tank, 1994; Brain and Bennett, 1997; Habets and Borst, 2005; Korogod et al., 2007). While the time course of Ca^{2+} binding to different proteins may seem short, it has been shown that a small increase in residual Ca^{2+} (hundreds of nM) can lead to a two-fold increase in the synaptic response, thereby prolonging the duration of plasticity significantly.

D3. Altered channel conductance

Another possibility that contributes to short-term effects includes alteration of presynaptic Ca^{2+} channel dynamics. Short-term depression is shown to be a result of decreased presynaptic Ca^{2+} entry following tetanic stimulation (Catterall and Few, 2008). Ca^{2+} sensing proteins (e.g. calmodulin, calcium-binding protein-1, etc.) can interact with Ca^{2+} channels to mediate activity-dependent changes, and reduce Ca^{2+} entry (Forsythe et al., 1998; Lee et al., 1999; Peterson et al., 1999; Lee et al., 2002; Lautermilch et al., 2005; Mochida et al., 2008). Calmodulin binding has also been shown to modulate voltage-gated Ca^{2+} channels and influence the size of the Ca^{2+} current itself (Inchauspe et al., 2004; Ishikawa et al., 2005; Catterall and Few, 2008). All of these features contribute to alterations in Ca^{2+} influx through presynaptic channels, which can directly affect neurotransmitter release.

D4. Vesicular pools

The vesicular pools at a given presynaptic axon terminal are also involved in short-term synaptic effects. The active zone of the terminal contains hundreds of vesicles, which are all clustered at different distances from the active zone, and are differentially released based on the strength of synaptic stimulation. The *readily releasable pool* of vesicles is immediately available when the presynaptic afferent is stimulated. The *recycling pool*, which includes about 10% of the terminal vesicles, is released with sustained high-frequency activation. The *reserve pool* is the remaining pool of vesicles and is not readily released, even with repeated stimulation. Therefore, based on the stimulus strength, there can be altered efficacy in synaptic release.

Posttetanic potentiation has been shown to result from an increase in the size of the readily releasable pool of vesicles, at some synapses (Habets and Borst, 2005). Contrastingly, longer lasting forms of depression are due to depletion of the recycling pool of vesicles. This theory states that high-frequency stimulation could deplete the recycling pool and replenishment from the reserve pool cannot keep up with vesicular loss during tetanic stimulation (Stevens and Wesseling, 1999; Catterall and Few, 2008).

1.7 Plasticity in the thalamocortical circuit

A. Receptive field reorganization

We know from previous studies that thalamocortical circuits are capable of expressing plasticity of receptive fields. Early primate studies show that ablation of primary sensory afferent produces somatotopic cortical reorganization (Merzenich et al., 1983). Following that study, Krupa et al. (1999) showed the differential effects of inhibiting corticothalamic feedback from the primary somatosensory cortex, versus primary sensory afferents onto relay neurons of the ventroposterior medial thalamus. Inactivation of corticothalamic inputs resulted in immediate changes in thalamic receptive field properties, which impacts sensory information transfer from primary sensory afferents, as well. These results show the dynamic equilibrium between the ascending sensory inputs and corticothalamic inputs in receptive field organization (Merzenich et al., 1983; Calford and Tweedale, 1988; Krupa et al., 1999; Parker and Dostrovsky, 1999; Fox et al., 2002; Chowdhury et al., 2004). These studies all provide evidence of the dynamic nature of the thalamocortical circuit at the receptive field level.

While these studies show the effects of corticothalamic and sensory afferent modulation in receptive field organization, understanding the basis of these effects at the

synaptic level is still unclear. Therefore, further studies regarding synaptic plasticity in the thalamus can bridge the gap between synaptic plasticity and how it manifests in altered receptive field reorganization.

B. Electrophysiological studies of plasticity in the thalamocortical circuit

Different forms of synaptic plasticity at the cellular level have been demonstrated at various thalamic synapses. One of the first studies that displayed long-term synaptic plasticity at corticothalamic synapses was shown by Castro-Alamancos and Calcagnotto (1999). Low frequency (10 Hz) tetanic stimulation of corticothalamic afferents produced Long-lasting potentiation of synaptic excitation in relay neurons, which was mediated by a presynaptic, NMDAR-independent mechanism. In a different study, LTP could be induced at corticothalamic synapses when paired with postsynaptic depolarization; however, this form of plasticity is NMDAR-dependent (Hsu et al., 2010).

There have been reports of plasticity at other thalamocortical synapses, including the thalamoreticular synapse and at inhibitory synapses. Pairing postsynaptic burst discharge in TRN neurons with synaptic activation of thalamoreticular afferents was found to produce LTP in TRN neurons (Astori and Luthi, 2013). These studies show how the communication between the TRN and relay nuclei can be enhanced with repeated burst activity, which could imply the role of spindle oscillations in strengthening intrathalamic circuitry during sleep.

Sieber et al. (2013) showed that LTP can be induced at inhibitory synapses from the TRN onto relay neurons in the posterior medial thalamic nucleus. Plasticity could be induced by postsynaptic burst discharge alone, and is triggered by NO release to induce enhanced GABAergic transmission (Sieber et al., 2013). The previously described studies

include the few reports of synaptic plasticity at thalamocortical circuits (Castro-Alamancos and Calcagnotto, 1999; Hsu et al., 2010; Astori and Luthi, 2013; Sieber et al., 2013), so it is evident that other synapses in the thalamocortical circuit need to be investigated to determine the role of plasticity in information processing. Additionally, while previous findings have examined LTP, there have been few reports of short-term plasticity in the thalamus. A shorter lasting change in synaptic efficacy could have major implications on the transfer of information through the thalamocortical sensory circuit. Therefore, these gaps in our knowledge of plasticity at synapses in the thalamocortical circuit need to be addressed to gain a more complete understanding of plasticity, involving both long and short-term changes, in the thalamus.

1.8 Concluding Remarks

The hypotheses that I set out to test in this dissertation address the state-dependent contribution to synaptic plasticity in the thalamus. I hypothesize that thalamic firing mode can influence information transfer at corticothalamic synapses in the thalamocortical circuit. I then set out to determine the cellular mechanism that contributes to altered synaptic transmission. My research will aid in forming a more integrated understanding regarding the role of plasticity at several synapses in the thalamocortical circuit, and the interrelated role that multisynaptic plasticity may play in affecting information transfer to the cortex. Novel findings from my research will contribute further to the growing field of plasticity in thalamocortical circuits. These findings can lead to conclusions regarding altered sensory information transfer to the cortex and how plasticity at the thalamic level could modulate corticocortical communication via the thalamus.

APPENDIX

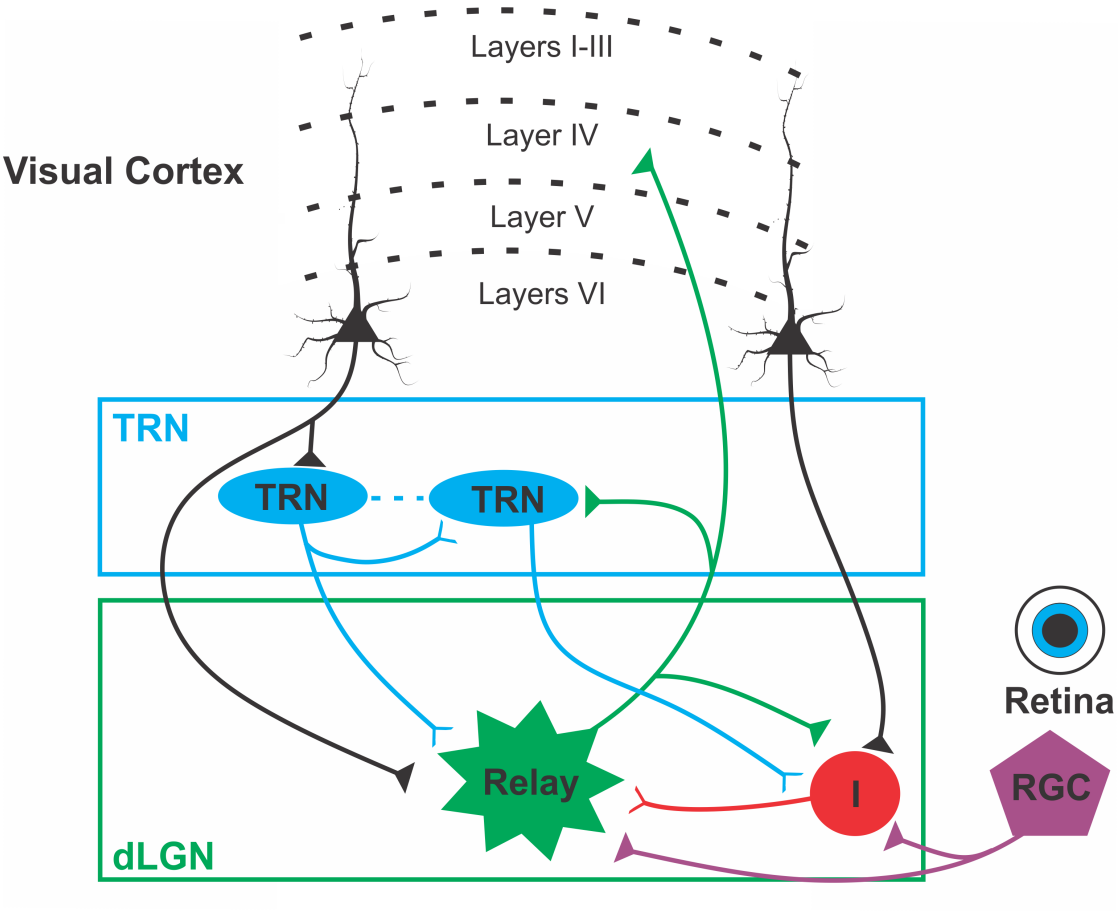

Figure 1.1. Thalamocortical circuit
Schematic of thalamocortical circuitry in the dLGN. Open synapses: inhibitory; solid synapses: excitatory.
I: Interneuron; Relay: Thalamocortical relay neurons; TRN: Thalamic reticular nucleus; RGC: retinal ganglion cell; PBR: parabrachial region.

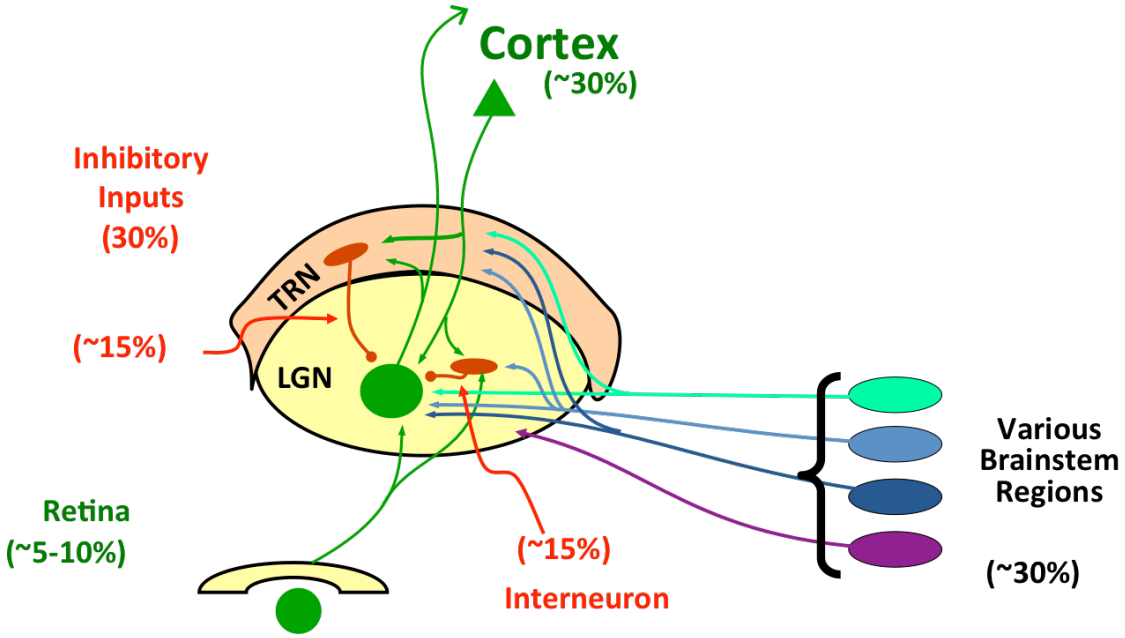

Figure 1.2. Schematic of inputs to dLGN
Afferents to the dLGN are represented as excitatory (green), from the cortex and retina, or inhibitory (red), from local circuit interneurons and TRN. Other inputs from brainstem regions account the remaining percentage of inputs.

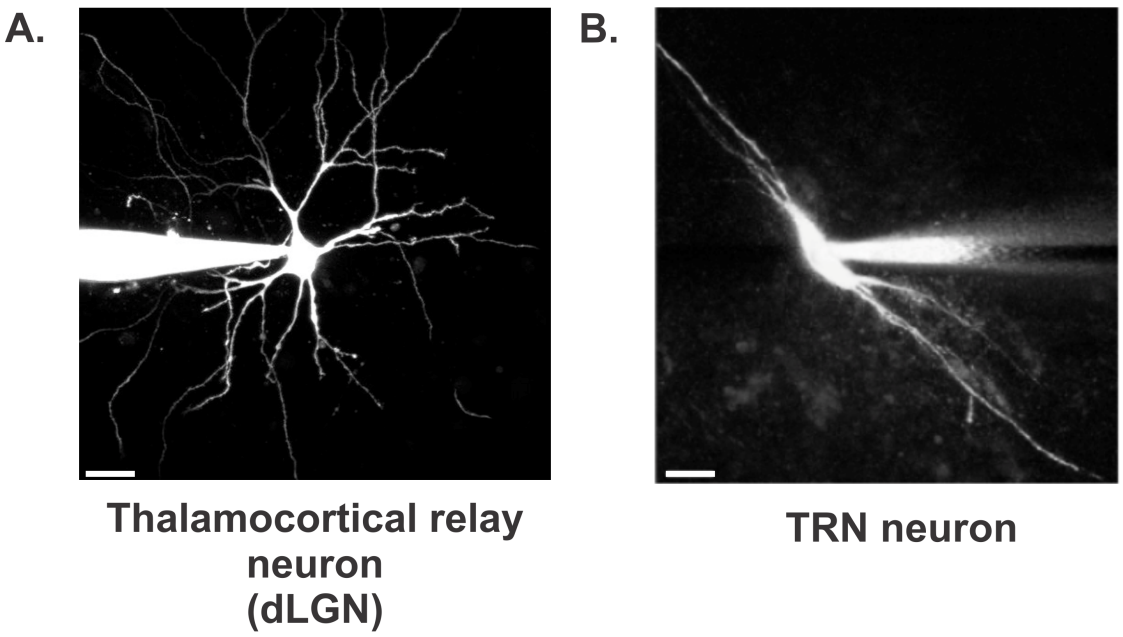

Figure 1.3. Thalamic neurons
Examples of two thalamic neurons filled with fluorescent indicator, Alexa 594 (25 μM), and imaged using 2PLSM.
A. Thalamocortical relay neuron from the dLGN. B. TRN neuron.
Scale bar = 20 μm.

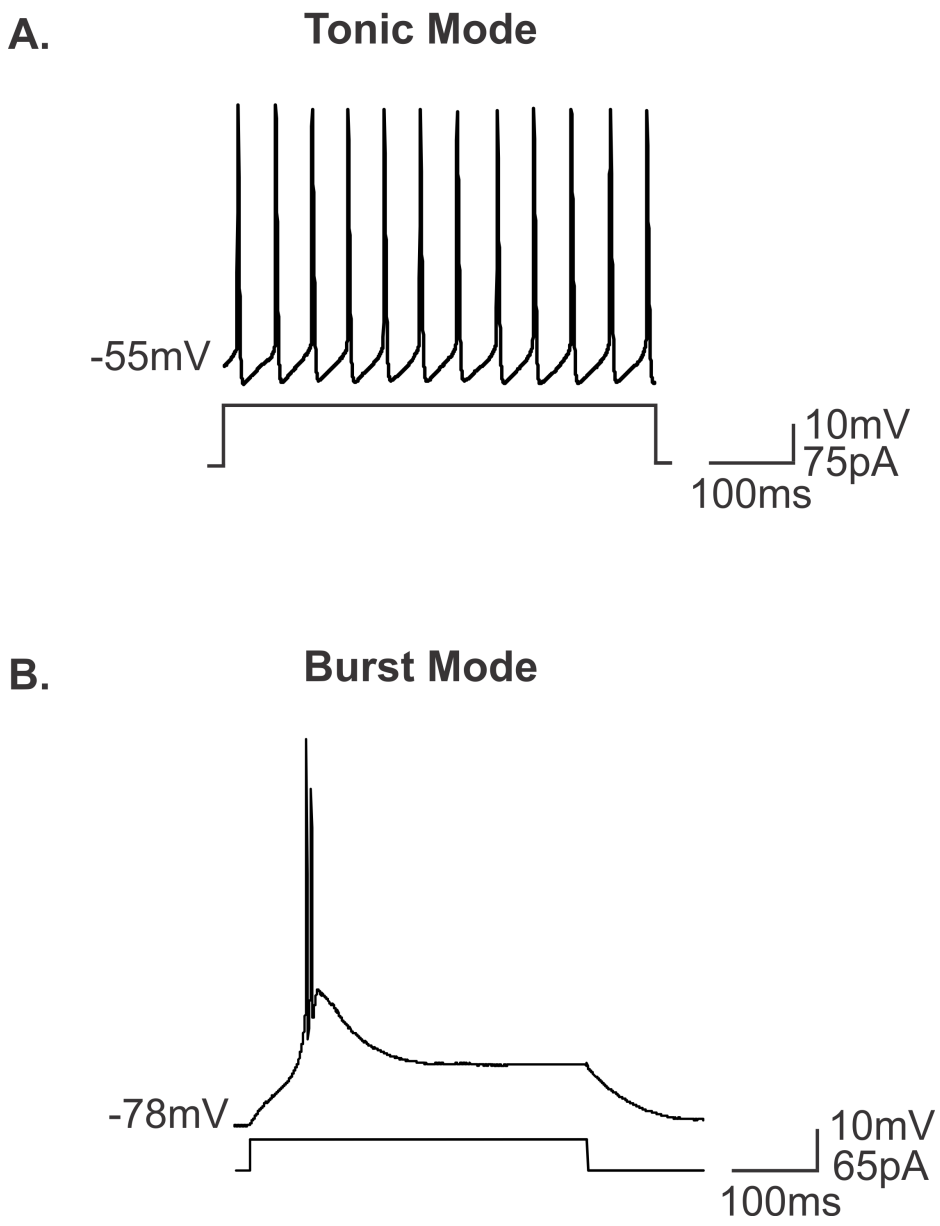

Figure 1.4. Thalamic firing modes
Representative traces of A. tonic and B. burst firing modes, recorded from thalamocortical relay neurons. Upper trace represents recorded membrane potential; lower trace represents injected current.

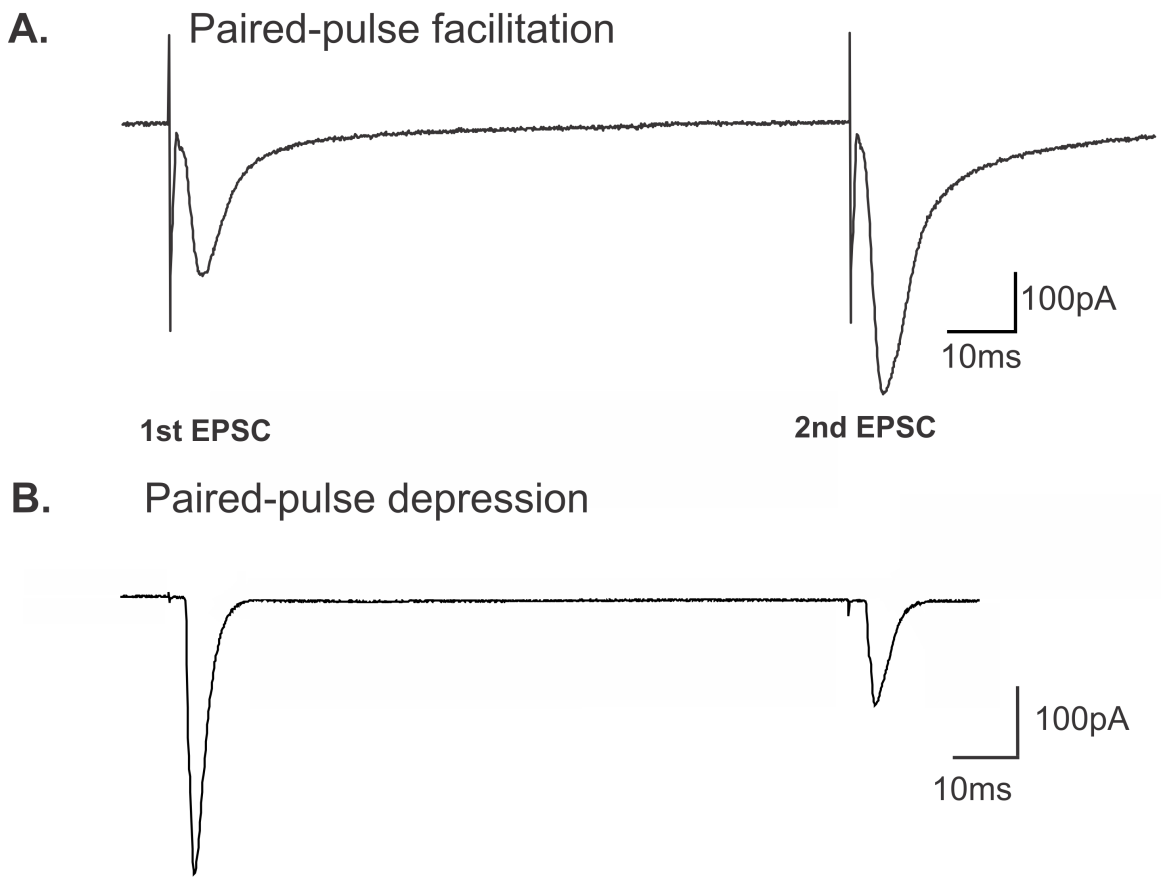

Figure 1.5. Paired pulse experiments
A. Paired-pulse facilitation at corticothalamic synapses. Representative trace of evoked EPSCs from a thalamocortical relay neuron, following stimulation (100 ms interstimulus interval) of corticothalamic afferents, via the internal capsule. B. Paired-pulse depression at retinogeniculate synapses. Representative trace of evoked EPSCs from a thalamocortical relay neuron, following stimulation of retinogeniculate afferents via the optic tract.

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CHAPTER II: STATE-DEPENDENT PLASTICITY AT CORTICOTHALAMIC SYNAPSES IN THALAMIC NEURONS

2.1 Introduction

Thalamocortical circuits are involved in the modulation and transfer of sensory information to the cortex. Thalamic relay neurons transfer information to the cortex differently based on their action potential firing mode (Jahnsen and Llinas, 1984b; Guido et al., 1995; Mukherjee and Kaplan, 1995; Swadlow and Gusev, 2001). The sensory information that is transferred through thalamocortical circuits enters the thalamus via primary sensory afferents, which convey information from various sensory modalities to the respective thalamic nucleus (e.g. retinal efferents to the dorsal lateral geniculate nucleus (dLGN); medial lemniscal efferents to ventrobasal nucleus (VB)). While primary sensory afferents are responsible for bringing the main sensory information to the thalamus, they only represent ~10% of synaptic contacts onto thalamic relay neurons. Corticothalamic synapses account for ~30-44% of inputs onto relay neurons (Liu et al., 1995; Erisir et al., 1997). Although corticothalamic afferents compose a large percentage of inputs to relay neurons, but their role in controlling information transfer through the thalamus has not been extensively elucidated. Synaptic plasticity in the thalamocortical circuit is of particular interest, since it could effectively be one mechanism through which corticothalamic afferents modulate sensory information transfer to the cortex for perception.

Thalamic neurons produce action potential discharge in one of two distinct modes: tonic and burst firing modes. The firing mode of the neuron is voltage dependent, and is mediated by the transient, low-voltage-activated T-type Ca^{2+} current, I_T. Tonic mode is the

predominant firing mode when I_T is inactivated by depolarized membrane potentials (> -60 mV), preventing Ca^{2+} entry through T-type Ca^{2+} channels. Tonic discharge is represented by repetitive action potential firing, with the frequency of discharge correlating linearly with the duration and intensity of the stimulus (Guido et al., 1992). At hyperpolarized membrane potentials (< -65 mV), T-type Ca^{2+} channels are deinactivated, and a suprathreshold stimulus will produce a low-threshold Ca^{2+} spike (LTS) with a transient, high frequency discharge of Na^+-dependent action potentials riding on its crest (Jahnsen and Llinas, 1984a, b; Suzuki and Rogawski, 1989; Zhan et al., 1999). Since the firing mode of thalamic relay neurons has been shown to affect the nature of information transfer to the cortex (Guido et al., 1995; Guido and Weyand, 1995; Swadlow and Gusev, 2001), the state of the neuron is a significant factor to consider when investigating thalamic information transfer.

Synaptic plasticity in the thalamocortical circuit could be a mechanism through which corticothalamic afferents modulate incoming sensory information. While some studies have investigated plasticity in the thalamocortical circuit or within intrathalamic connections (Steriade and Timofeev, 1997; Granseth and Lindstrom, 2004), most of the seminal studies have focused on transient changes in synaptic efficacy based on repetitive afferent activation, which can last for hundreds of milliseconds to seconds (Frigyesi, 1972; Steriade and Wyzinski, 1972; Lindstrom and Wrobel, 1990; Steriade and Timofeev, 1997). More recently, long-term plasticity in the thalamus has been identified at corticothalamic synapses (Castro-Alamancos and Calcagnotto, 1999; Hsu et al., 2010), and thalamoreticular synapses (Astori and Luthi, 2013).

Studies of thalamic long-term plasticity have used a variety of stimulation paradigms including presynaptic tetanic stimulation (Castro-Alamancos and Calcagnotto, 1999), postsynaptic stimulation (Sieber et al., 2013), or coincident pre- and postsynaptic stimulation (Hsu et al., 2012; Astori and Luthi, 2013). Coincident pre- and postsynaptic stimulation can induce a summation of dendritic Ca^{2+} transients, which can produce differential synaptic plasticity based on the magnitude of dendritic Ca^{2+}(Camire and Topolnik, 2014). Additionally, studies of spike-timing dependent plasticity in various other brain regions have shown pairing a synaptic response with backpropagation of action potentials, induced by somatic current injection, also produces summation of dendritic Ca^{2+} transients. In this stimulation paradigm, the magnitude of Ca^{2+}, determined by the timing of pre- and postsynaptic stimulation, can lead to the production of synaptic potentiation or depression (Stuart and Sakmann, 1994; Spruston et al., 1995; Magee and Johnston, 1997; Koester and Sakmann, 1998; Dan and Poo, 2004). Therefore, since coincident stimulation models have been effective in producing synaptic plasticity at various brain synapses based on Ca^{2+}-dependent dynamics, we wanted to determine if plasticity could be induced in thalamic neurons via coincident pre- and postsynaptic stimulation paradigms.

Since relay neurons fire action potentials in two different firing modes, which are Ca^{2+}-dependent, we wanted to test the hypothesis that the firing mode of the postsynaptic neuron can affect plasticity at corticothalamic synapses. In the stimulation paradigm, burst or tonic discharge will be induced in the postsynaptic cell, and closely followed by electrical stimulation of corticothalamic afferents. Since burst mode is mediated by a transient Ca^{2+} influx in the postsynaptic neuron, we hypothesize that larger dendritic Ca^{2+} transients during paired presynaptic stimulation with postsynaptic burst discharge will be more

likely to induce synaptic potentiation than when corticothalamic afferent activation is paired with tonic discharge in the postsynaptic cell.

We will be looking at plasticity in thalamocortical relay neurons and thalamic reticular nucleus (TRN) neurons. The TRN is the major inhibitory nucleus of the thalamus, which plays a significant role in modulating information transfer to the cortex (Steriade and Deschenes, 1984; Huguenard and Prince, 1994; Cox et al., 1997). Therefore, we wanted to determine the contribution of plasticity at corticothalamic synapses to relay nuclei, as well as to the TRN.

In addition to the corticothalamic synapses, we wanted to determine if plasticity is present at sensory synapses, as well. Primary sensory afferents bring the main sensory information into the thalamus, and plasticity at these synapses could change the nature of information that is entering the brain. Identifying synaptic plasticity at different thalamic nuclei can lead to a more comprehensive understanding of the expression of plasticity in the thalamocortical circuit, which can lead to conclusions about its functional significance.

2.2 Materials and Methods

All experimental procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Michigan State University Institutional Animal Care and Use Committee. We used the minimal number of animals necessary to complete these experiments.

Thalamic brain slices were prepared from Sprague Dawley rats (postnatal age: 15-25 days) of either sex. Briefly, animals were deeply anesthetized with isoflurane (2-4% inhalation), perfused with cold, oxygenated slicing solution, and quickly decapitated. Brains were removed and placed in cold (~4°C), oxygenated (95% O_2, 5% CO_2) slicing solution

containing the following (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgSO_4$, 0.5 $CaCl_2$, 26.0 $NaHCO_3$, 10.0 glucose, and 234.0 sucrose. Thalamic slices (300-400 μm) were cut using a vibrating tissue slicer in the coronal plane or modified parasagittal plane (Turner and Salt, 1998) for dLGN recordings, or the horizontal plane for VB and TRN recordings. Slices were transferred to a warmed (~35°C) holding chamber with oxygenated (5% CO_2, 95% O_2) physiological saline containing the following (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose. After 20-30 minutes, the holding chamber was reduced to room temperature (~25°C), and slices were incubated for an additional 60 minutes before recording.

For recording, individual slices were transferred to a recording chamber that was maintained at 30°C with oxygenated (95% O_2, 5% CO_2,) physiological saline (2.5-3 ml/min). Individual relay neurons were identified using a microscope, equipped with differential interference contrast optics and 63x water-immersion objective (Zeiss Axioexaminer.A1). Recording pipettes were made from 1.5mm OD borosilicate glass and had a tip resistance of 2-6 MΩ when filled with the following internal solution (in mM): 117 K-gluconate, 13.0 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2-ATP, and 0.4 Na-GTP (pH 7.3, 290 mOsm). The internal solution resulted in a junction potential of ~10 mV and was corrected for in all voltage recordings. During recordings, pipette capacitance was neutralized, and the access resistance was continually monitored. Recordings typically had access resistances <20 MΩ. In addition, input resistance and holding current were continuously monitored throughout the experiment.

Recordings were made using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Voltage-clamp recordings were collected at a holding potential of -60 mV,

and current-clamp recordings were recorded at resting membrane potential (~-68 mV). Excitatory postsynaptic currents or potentials (EPSC/Ps) were evoked by electrical stimulation of respective afferents with a bipolar tungsten stimulating electrode. Stimulation of the corticothalamic afferents was achieved by placing the stimulating electrode in the optic radiation (for dLGN recordings) or internal capsule (for VB and TRN recordings). Stimulation of retinogeniculate afferents was achieved by placing the stimulating electrode in the optic tract for dLGN recordings.

All experiments were done in the presence of $GABA_A$ and $GABA_B$ antagonists, 6-Imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid hydrobromide (SR-95531 hydrobromide; 10μM) and *N*-[[*trans*-4-[[(4-Amino-2-quinazolinyl)amino]methyl]cyclohexyl]methyl]-1-naphthalenesulfonamide hydrochloride (CGP-54626 hydrochloride; 5μM) respectively, to isolate EPSCs. Antagonists (Tocris) were bath applied for 5-10 min before subsequent experimental tests.

A. Synaptic stimulation

Excitatory postsynaptic currents and potentials (EPSC/Ps) were evoked (duration: 100 μs, intensity: 50-500 μA) at 15-second intervals and 30 consecutive, stable responses were recorded prior to the induction protocol (*below*). Neurons were recorded in voltage clamp mode with a V_{hold}: -60 mV prior to, and following the induction protocol. For corticothalamic stimulation, the stimulating electrode was placed in the optic radiation for dLGN recordings (**Fig. 2.1A**), or the internal capsule for VB recordings (**Fig. 2.6A**).

B. Induction paradigm

The *paired burst stimulation* protocol involved pairing a postsynaptic burst with corticothalamic stimulation. To induce bursting, the membrane potential of the cell was

adjusted to -80 mV (in current clamp mode) and an intracellular somatic current injection was given (current step: ~350 pA, duration: 35 ms). Burst stimulation was closely followed (5 ms latency) by single stimulation of the corticothalamic afferents to evoke an EPSP. The pairing was administered 400 times at 2 Hz (**Fig. 2.1B**).

The *paired tonic stimulation protocol* involved pairing a postsynaptic action potential with corticothalamic stimulation. To induce single tonic action potential firing, the membrane potential of the cell was maintained at -60mV and an intracellular somatic current step was given (1000 pA, 2 ms). Tonic stimulation was closely followed (5 ms latency) by single corticothalamic stimulation to evoke an EPSP. The pairing was administered 400 times at 2 Hz (**Fig. 2.1B**).

The *paired burst + train* and the *paired tonic + train* protocols involved the pairing of either a burst or single action potential in the postsynaptic cell (as described above), with a train of corticothalamic stimuli (100 Hz, 5 pulses) in place of the single corticothalamic EPSP (**Fig. 2.6A**). The unpaired train stimulation protocol involved brief tetanus (100 Hz, 5 pulses), which was repeated 400 times at 2 Hz (**Fig. 2.7A**).

C. Retinogeniculate stimulation

For experiments involving retinogeniculate activation, EPSCPs were evoked by electrical stimulation of retinogeniculate afferents by placing the stimulating electrode in the optic tract (**Fig. 2.5A**), and the neurons were recorded at resting membrane potential (~-68 mV). Either the paired burst stimulation or paired tonic stimulation protocols were performed, as described above, and paired with optic tract stimulation in place of corticothalamic stimulation.

D. Glutamate Uncaging

Glutamate uncaging and whole-cell recordings were simultaneously performed using a two-photon laser scanning microscope (2PLSM) (Ultima; Bruker Technologies) coupled with a tunable infrared laser (MaiTai HP; Spectra Physics) to visualize neurons. A fixed wavelength, 1-photon laser (405 nm; 100 mW Coherent CUBE diode laser) was used to uncage glutamate onto a dendrite. Patch pipettes contained the fluorescent indicator, Alexa Fluor 594 (25 μM), and after the whole-cell configuration was attained, sufficient time was allowed for diffusion of the indicator throughout the cell and dendritic arbor (~20 min). Subsequently, imaging was performed using laser excitation (820 nm), with a high numerical aperture water immersion objective (60x; Olympus). Once the neuron and dendrites were visualized, a distal dendritic location was chosen (> 150 μM from the soma). For glutamate uncaging, a separate puffing pipette was filled with 4-methoxy-7-nitroindolinyl-caged-L-glutamate (MNI glutamate; 5 mM), and positioned near the distal dendritic location. MNI glutamate was released from the puffing pipette using a pressurized valve (0.75-3 psi, 50 ms duration), and photoactivation of MNI glutamate was achieved by focusing the 1-photon laser coupled into the scan head with a photoactivation module with a set of galvometers controlled by TriggerSync software, at the dendrite. Short bursts of 5 focal excitations (0.1 ms interval) produced a transient glutamate depolarization (e.g. **Fig. 2.4B**).

Glutamate depolarizations were recorded in current clamp mode (at resting membrane potential, ~ -68 mV) by uncaging MNI glutamate at distal dendritic locations (10 second intervals) to collect baseline amplitudes. Either the paired burst or paired tonic stimulation protocols were administered. For the paired burst protocol, the relay neuron

was hyperpolarized to approximately -80 mV, and a somatic current injection (current step: 350 pA, duration: 35 ms) produced a burst discharge, which was closely followed by a glutamate-induced depolarization (latency: 35 ms). This pairing was repeated 100 times, at a frequency of 0.2 Hz. For the paired tonic stimulation protocol, the neuron was depolarized to -60 mV, and a somatic current injection (current step: 1000 pA, duration: 2 ms) produced a single action potential, which was closely followed by a glutamate-induced depolarization. Following either stimulation protocol, the change in amplitude of glutamate depolarizations was recorded and compared to baseline measurements.

Data acquisition and analyses were performed using pCLAMP software (Molecular Devices). Data were digitized at 10 kHz, filtered online at 2.5 kHz, and stored on a computer. Pre- and post-induction EPSC/P amplitudes were compared, and cells were classified into groups that displayed either facilitation, depression, or unaltered synaptic responsiveness based on a 95% confidence interval. Facilitation was defined by a 2x standard deviation (SD) increase in EPSC/P amplitude that persisted for at least 10 consecutive responses (2.5 min). Depression was defined by a 2x SD decrease in EPSC/P amplitude that persisted for at least 10 consecutive responses. After grouping the cells into different categories of change, cells were compiled and normalized to 1 to form population data.

Magnitude changes from baseline were taken using the first 5 events following the stimulation protocol, or at peak amplitude. Comparisons based on magnitude were made using a Student's *t* test, comparing the post-induction peak amplitudes following different stimulation protocols (unpaired *t* test). One-way ANOVAs were used to examine magnitude differences in groups that underwent different induction protocols (between groups

factor). Data are presented as Mean ± SEM, and statistical significance was assessed with *p* value < 0.05.

2.3 Results

For these experiments, whole-cell intracellular recordings were obtained from 269 thalamocortical relay neurons and 79 TRN neurons.

A. Thalamocortical relay neurons display state-dependent synaptic plasticity at corticothalamic synapses in dLGN following paired stimulation

We first tested the hypothesis that corticothalamic synapses display state-dependent plasticity in dLGN relay neurons. We initially tested if pairing of a postsynaptic burst discharge with corticothalamic afferent stimulation (400 pairings at 2 Hz) would alter the resulting EPSC (**Fig. 2.1B,** Burst State). Following the paired burst stimulation protocol, 41% of cells (18 of 44) tested showed facilitation of EPSC amplitude, 36% (16 of 44) displayed depression, and 23% (10 of 44) showed unchanged EPSC amplitude following paired stimulation (**Fig. 2.2A**). In the 18 cells, which showed short-term facilitation of the EPSC, the peak effect (73.6 ± 7.4% increase) occurred immediately following the induction protocol and returned to baseline levels within 10-15 minutes. In contrast, the depression of the EPSC observed in the different subpopulation of neurons was less robust compared to the facilitation (24.9 ± 7.1% initial decrease), however, the suppression lasted for the duration of the experiment (>20 minutes), and increased in magnitude over time (36.6 ± 5.9% decrease at 20 minutes post induction). To determine if the pairing was required for plasticity, we carried out the experiments with corticothalamic stimulation alone, or postsynaptic burst discharge alone. Single corticothalamic EPSPs (400 repetitions at 2 Hz) did not alter EPSC amplitude (**Fig. 2.2B**; n = 12). Similarly, postsynaptic

burst discharge alone (400 repetitions at 2 Hz) did not alter EPSC amplitude (**Fig. 2.2C**; n = 6). These findings show that pairing is necessary to induce synaptic plasticity.

For the next set of experiments, tonic firing was paired with corticothalamic stimulation to determine if it would alter synaptic responsiveness. The paired tonic stimulation protocol consisted of single action potentials paired with a corticothalamic EPSP (400 pairings at 2Hz), as illustrated in **Fig. 2.1B** (Tonic State). Following the paired tonic stimulation protocol, only 17% of cells (5 of 30) showed facilitation of the EPSC. In these cells, the magnitude of facilitation (36.5 ± 10.3%) was significantly lower than that observed following the paired burst protocol ($p = 0.001$, unpaired *t* test), and facilitation only lasted for <10 minutes before recovering to baseline. In contrast, the majority of neurons (77%: 23 of 30) responded with depression of the EPSC amplitude (**Fig. 2.3A**). The magnitude of depression (57 ± 4.7%) was significantly greater than that seen following paired burst stimulation ($p < 0.0001$, unpaired *t* test). However, while the depression showed trends towards baseline levels over the time course of the experiment, it remained at depressed levels for the remainder of the experiment (>20 minutes). To determine if tonic firing alone was sufficient to induce plasticity, the unpaired control experiment was performed. Single action potentials were delivered (400 repetitions at 2 Hz), and no change in synaptic efficacy was observed (**Fig. 2.3C**; n=5). In summary, these results suggest a state-dependent effect following paired stimulation. Paired burst discharge produced more facilitation, and paired tonic firing yielded more depression (**Table 2.1**).

Table 2.1: EPSC responses in dLGN relay neurons following paired burst or tonic corticothalamic stimulation

Corticothalamic Stimulation (dLGN relay neurons)	**Burst + CT single** (n=44)	**Tonic + CT single** (n=30)	**CT single alone** (n=12)	**Burst alone** (n=6)	**Tonic Alone** (n=5)
Increase	41% (n=18) 73.6±7.4% increase	17% (n=5) 36.5±10.3% increase	0	0	0
Decrease	36% (n=16) 24.9±7.1% decrease	77% (n=23) 57±4.7% decrease	0	0	0
No Change	23% (n=10)	6% (n=2)	100% (n=12)	100% (n=6)	100% (n=5)

B. Plasticity is produced following paired burst discharge with glutamate-induced depolarizations

We showed that paired stimulation with a corticothalamic EPSP is capable of inducing plasticity, so next, glutamate uncaging was utilized to test if the observed plasticity requires coactivation of pre- and postsynaptic components by replacing synaptic activation with uncaging glutamate to activate postsynaptic receptors. These experiments effectively isolate all manipulations to the postsynaptic neuron. The objective of this experimental manipulation was to mimic corticothalamic afferent activation; therefore, glutamate was uncaged at distal dendritic locations, since corticothalamic projections primarily synapse onto distal dendrites of relay neurons (Liu et al., 1995).

First, the paired burst stimulation protocol using glutamate uncaging was performed (100 repetitions at 0.2 Hz), as detailed in **Fig. 2.4A.** 100 repetitions was used for this experimental paradigm, due to limitations of the glutamate uncaging technique. Repeated stimulation caused global membrane depolarization, so fewer stimulus repetitions were used to prevent this from occurring. Following the stimulation protocol,

we observed that 33% of all cells (9 of 27) displayed plasticity. 19% of all cells (5 of 27) showed facilitation, and 15% of cells (4 of 27) showed depression in amplitude following the stimulation protocol (**Fig. 2.4B**). Interestingly, cells that displayed facilitation did not show recovery over the course of the experiment (>10 minutes). However, the cells that showed depression in glutamate depolarization amplitude recovered to baseline levels in <10 minutes. Next, we wanted to compare these results with those following pairing of tonic stimulation with glutamate depolarizations.

Following paired tonic stimulation with glutamate depolarizations, all cells displayed unchanged amplitude of glutamate-induced responses (**Fig. 2.4C;** n = 4). Since paired bursting produced facilitation or depression in the amplitude of glutamate depolarizations, this suggests that postsynaptic modifications may be contributing to the plasticity observed at corticothalamic synapses.

C. Retinogeniculate synapses do not display plasticity following paired postsynaptic firing with single EPSP in dLGN

Next, we wanted to determine if the plasticity that we observed with corticothalamic stimulation was unique to this pathway or a general feature of excitatory afferents on thalamocortical neurons. For these experiments, the stimulation electrode was placed in the optic tract to stimulate retinogeniculate fibers (**Fig. 2.5A**). After establishing a stable amplitude EPSP for at least 5 minutes, we initiated the paired burst and retinogeniculate stimulation protocol. Unlike the experiments with paired burst firing with a corticothalamic EPSP, the EPSP amplitude was unaltered in these experiments (**Fig. 2.5B;** n = 17). Next, we determined if paired tonic stimulation would alter the EPSP amplitude, and similar to the paired burst protocol, the EPSP amplitude was unaltered in all cells

tested (**Fig. 2.5C**; n = 21). These data indicate that the retinogeniculate pathway does not display synaptic plasticity following paired burst or tonic stimulation as we had observed with the corticothalamic pathway stimulation.

D. Corticothalamic synapses in VB display facilitation following paired postsynaptic firing with tetanic activation

Since we demonstrated that plasticity specifically occurs at corticothalamic synapses, we next wanted to determine if the magnitude or duration of plasticity could be enhanced by increasing the intensity of stimulation. In the following experiments we altered the paired induction protocol to replace the single corticothalamic stimulation with a brief tetanus (100 Hz, 5 pulses, **Fig. 2.6A**).

In addition, the horizontal slice preparation was used to record from VB thalamocortical relay neurons while stimulating the internal capsule, because this slice orientation retains more reliable corticothalamic connectivity. We tested the paired stimulation protocol in a small subset of VB neurons, to verify similar trends in plasticity, as observed in dLGN relay neurons.

The paired burst + train protocol consisted of a burst discharge paired with tetanic stimulation of corticothalamic afferents (5 pulses delivered at 100 Hz), with pairing repeated 400 times at 2 Hz, as detailed in **Fig. 2.6A** (Burst State). Following the paired burst + train protocol, nearly all cells (94%; 44 of 47 cells) tested showed a robust facilitation of EPSC amplitude (144.8 ± 10.1%). The amplitude of facilitation decreased over time, but still remained potentiated from baseline (17.7± 8.8%) for the remainder of the experiment (**Fig. 2.6B**). The remaining three cells displayed unaltered EPSC amplitude following stimulation. The addition of the repeated tetanic stimulation of corticothalamic

fibers significantly altered the probability of facilitation from 41 to 94%. Furthermore, with tetanic stimulation, we did not observe any suppression, as was seen following the single corticothalamic stimulation (16%).

We next tested if tonic firing paired with tetanic stimulation of corticothalamic afferents would alter EPSC amplitude. The paired tonic + train protocol consisted of a single action potential paired with a train of EPSPs (**Fig. 2.6A**, Tonic State). Following tonic + train stimulation, nearly all cells (93%; 14 of 15) displayed facilitation of the EPSC amplitude (156.4 ± 18.0%) that decreased over time, but still remained at a potentiated level (30.4 ± 12.8%) for the remainder of the experiment (>12 minutes) (**Fig. 2.6C**). The magnitude of initial potentiation following paired tonic + train stimulation was not significantly different from potentiation seen following paired burst discharge + tetanic stimulation (p = 0.625, unpaired t test). Interestingly, paired tonic stimulation primarily produced depression in EPSC amplitude, but paired tonic + train stimulation only showed facilitation of the EPSC. Because both paired tonic and burst firing with tetanic stimulation produce facilitation in EPSC amplitude, we next determined if the common factor, tetanic stimulation, is sufficient to induce plasticity when unpaired.

E. Tetanic stimulation of corticothalamic afferents produces short-term facilitation in VB

The unpaired train stimulation protocol consisted of a series of brief tetanic (5 pulses at 100 Hz), repeated 400 times at 2 Hz (**Fig. 2.7A**). Following tetanic stimulation, 81% of cells (34 of 42) displayed facilitation of EPSC amplitude (108.6 ± 7.7%), which recovered to baseline after 15-20 minutes (**Fig. 2.7B**). Since facilitation resulted from both paired and unpaired tetanic stimulation, the observed facilitation for respective stimulation protocols

was compared (**Fig. 2.7C**). These data show that the peak magnitude of facilitation produced by paired burst + train and paired tonic + train did not significantly differ; however, the facilitation produced by unpaired tetanic stimulation was significantly lower ($F_{2,14}$ = 9.37; p = 0.004). These results indicate that most of the facilitation can be attributed to tetanic activation of corticothalamic afferents, but when paired with postsynaptic firing, pairing contributes to a greater magnitude of facilitation (**Table 2.2**).

Table 2.2: EPSC responses in VB relay neurons following paired and unpaired tetanic corticothalamic stimulation

Corticothalamic Stimulation (in VB relay neurons)	**Burst+CT train** (n=47)	**Tonic+CT Train** (n=15)	**Unpaired Train** (n=42)
Increase	94% (n=44) 144.8±10.1% increase	93% (n=14) 156±18% increase	81% (n=34) 108.6±7.7% increase
Decrease	0	0	0
No Change	6% (n=3)	7% (n=1)	19% (n=8)

F. Retinogeniculate synapses display depression in dLGN following tetanic stimulation

In light of the alterations seen when tetanic stimulation was introduced to the stimulation protocol at corticothalamic synapses, we wanted to determine if alterations in EPSC amplitude could be seen at retinogeniculate synapses following tetanic stimulation. Interestingly, tetanic stimulation of retinogeniculate synapses produced depression (84.8 ± 4.2%) in EPSC amplitude, that lasted > 17 minutes (**Fig. 2.8;** n = 11). Our previous findings showed that postsynaptic stimulation paired with single retinogeniculate EPSPs was not sufficient to produce plasticity, but tetanic stimulation can now induce lasting depression at retinogeniculate synapses. These findings show that the retinogeniculate pathway can display plasticity, but high frequencies are required to induce depression (**Table 2.3**).

Table 2.3: EPSC responses in dLGN relay neurons following paired and unpaired retinogeniculate stimulation

Retinogeniculate Stimulation (in dLGN relay neurons)	**Burst + OT single** (n=17)	**Tonic + OT single** (n=21)	**Unpaired tetanic stimulation** (n=11)
Increase	0	0	0
Decrease	0	0	100% (n=11) 84.8±4.2% decrease
No Change	100% (n=17)	100% (n=21)	0

G. Paired burst stimulation at corticothalamic synapses induces depression in TRN neurons

Corticothalamic afferents project to both relay nuclei, as well as TRN. In the intact circuit, activation of corticothalamic afferents will also impact TRN modulation of relay neurons. Therefore, determining whether plasticity can be induced in TRN neurons is imperative to our understanding of plasticity following corticothalamic activation.

Our previous data demonstrate that a single corticothalamic EPSP with coincident postsynaptic burst discharge induces the greatest percentage and magnitude of short-term facilitation in thalamic relay neurons. We have also shown that tetanic activation of corticothalamic synapses is sufficient to induce short-term facilitation, albeit at a lower percentage and magnitude than paired tetanic stimulation. Next, we wanted to determine if the same trends of plasticity could be observed in TRN neurons.

For these experiments, the horizontal slice preparation was used to stimulate the internal capsule and record from TRN neurons. The stimulation protocol was performed by producing a burst discharge followed by a single EPSP, from internal capsule activation (400 pairings at 2 Hz) (**Fig. 2.9A**, Burst State). Following the paired burst protocol, 47% of

cells (18 of 38) display depression (39 ± 5.9%) that lasted through the remainder of the experiment (>12 minutes), while the EPSCs were unaltered in the remaining neurons (**Fig. 2.9B,** upper panel). Interestingly, there was no facilitation of EPSC amplitude observed in TRN neurons following the paired burst protocol. We performed unpaired control experiments in the TRN, as well, to verify that paired stimulation was necessary to produce plasticity. We showed that burst discharge alone (n = 2, data not shown), or single corticothalamic EPSPs (n = 5, data not shown), both delivered at 2 Hz for 400 repetitions, were not sufficient to produce plasticity at corticoreticular synapses, showing the necessity of pairing in TRN neurons, as well.

Next, since adding tetanic stimulation enhanced the magnitude and duration of plasticity in relay neurons, we wanted to determine if depression in TRN neurons could be enhanced by pairing burst discharge with tetanic stimulation. For these experiments, burst discharge was paired with a train of EPSPs (5 pulses delivered at 100 Hz), and the pairing was repeated 400 times at 2 Hz. Following burst + train stimulation, 70% of cells (14 of 20) showed depression in EPSC amplitude that recovered in ~10 minutes, 25% of cells (5 of 20) showed unaltered EPSC amplitude, and just 1 cell showed facilitation (**Fig. 2.9B,** lower panel). The magnitude of depression that was seen following burst + train stimulation (54.6 ± 6.6%) was significantly greater than the depression following burst discharge paired with a single EPSP ($p < 0.0001$, unpaired *t* test).

Finally, we wanted to determine if unpaired tetanic stimulation of the internal capsule produced different results, since trains were sufficient to induce plasticity in relay neurons. Tetanic stimulation (5 pulses delivered at 100 Hz) of the internal capsule was repeated 400 times at 2 Hz. Following stimulation, 57% of cells (8 of 14) displayed

plasticity. Of these, 28.5% of cells (4 of 14) showed facilitation (135.6 ± 45.4%), and 28.5% of cells (4 of 14) showed depression (58.0 ± 10.24%) of EPSC amplitude. The remaining 43% of cells showed unaltered EPSC amplitude following tetanic stimulation (**Fig. 2.9C, Table 2.4**). These data were particularly interesting, since the unpaired tetanic stimulation protocol was able to produce facilitation; however, a lower percentage of cells displayed depression following unpaired stimulation than either of the paired stimulation protocols. These findings could lead to conclusions about the role of corticothalamic activation paired with burst discharge in the TRN, which primarily produces depression in these neurons.

Table 2.4: EPSC responses in TRN neurons following paired and unpaired corticoreticular stimulation

Internal Capsule Stimulation (in TRN neurons)	**Burst+IC single** (n=38)	**Burst+IC Train** (n=20)	**Unpaired Train** (n=14)
Increase	0	0	28.5% (n=4)
Decrease	47% (n=18) 39±5.9% decrease	70% (n=14) 54.6±6.6%	28.5% (n=4)
No Change	53% (n=20)	25% (n=5)	43% (n=6)

2.4 Discussion

Our findings show that there are state-dependent alterations in synaptic plasticity at different thalamic nuclei. Since the environment is constantly changing, the thalamocortical sensory circuit may induce synaptic alterations, so that incoming sensory information can be modulated, based on corticothalamic activation and thalamic firing mode. We show potentiation and depression of EPSCs, lasting at least 15-20 minutes, which can significantly affect information transfer through the thalamocortical circuit. Following facilitation in relay neurons, we observe a recovery to baseline levels of synaptic activity, demonstrating short-lasting effects. Short-term facilitation in relay neurons

potentially demonstrates an inducible thalamocortical circuit that can effectively transfer information, yet transiently alter information flow based on the current environmental demands.

We showed that in relay neurons, pairing postsynaptic burst discharge with activation of corticothalamic afferents causes a greater percentage of cells displaying facilitation of synaptic efficacy, while paired tonic firing produces more depression of synaptic activity. This state-dependent effect is novel, especially since other studies show the absence of any type of plasticity with tonic firing (Hsu et al., 2012; Astori and Luthi, 2013; Sieber et al., 2013). Together, our findings show that the firing mode can biphasically determine how corticothalamic modulation will affect future incoming sensory information. The corticothalamic feedback afferents could be exerting a gain control mechanism based on relay neuron firing mode, as a means of increasing the transmission of the sensory signal, while decreasing noise in the circuit. One theory may involve the behavioral implications of tonic firing, which occurs in alert, awake states (Steriade et al., 1986; Guido and Weyand, 1995; Ramcharan et al., 2000; Sherman, 2001). If thalamocortical information transfer is already heightened in tonic mode, the corticothalamic synapses might be compensating for this enhanced state of information transfer by dampening excitatory feedback. Studies have shown the impact of enhanced cortical excitatory feedback, which may introduce excessive interference or noise in the thalamocortical circuit (Wolfart et al., 2005). Additionally, tonic firing mode has also been shown to be accompanied with more spontaneous activity compared to burst mode (Guido et al., 1995). Corticothalamic-induced depression could act to quiet over-excitation, which may impede efficient information flow to the cortex. Contrastingly, in burst mode, we observe similar

percentages of cells showing facilitation and depression of synaptic activity. When the thalamocortical neuron is in burst mode, the cortical drive could be transiently heightened or depressed to improve transmission of more salient sensory information.

The duality of Ca^{2+}-dependent firing modes in thalamic neurons could contribute to differential dendritic Ca^{2+} transients when paired with synaptic activation, which could account for our results. Postsynaptic burst discharge causes enhanced dendritic Ca^{2+} due to backpropagation of action potentials and the influence of I_T (Stuart and Sakmann, 1994; Spruston et al., 1995; Magee and Johnston, 1997; Larkum et al., 1999; Williams and Stuart, 2000; Crandall et al., 2010; Errington et al., 2010). When coincident presynaptic activity is paired with burst discharge, supralinear Ca^{2+} levels may be summating at the dendrites to produce an enhanced synaptic response (Koester and Sakmann, 1998). While tonic firing can facilitate action potential backpropagation, this firing mode does not activate as large of a dendritic Ca^{2+} current in the dendrites, since I_T does not significantly contribute to Ca^{2+} influx in tonic mode (Crandall et al., 2010; Sieber et al., 2013); this could produce sublinear Ca^{2+} levels, leading to suppression of synaptic activity.

The dendritic Ca^{2+} levels could also explain the different responses that we see within each population that received either paired burst or paired tonic stimulation. Each experimental group contained cells that showed increases, decreases, or unchanged EPSC amplitude. It is possible that the relative strength of the burst in a particular relay neuron, depends on the morphology and dendritic branching, which can impact how backpropagating action potentials may infiltrate the dendrites (Williams and Stuart, 2000). If a particular relay neuron has a greater number of activated T-type Ca^{2+} channels, and therefore, produces a stronger burst, there may be a greater likelihood of producing

supralinear Ca^{2+} levels in the dendrites. Similarly, if a relay neuron has comparatively less dendritic branching, it may be more likely to send the signal through all of the dendrites based on active dendritic conductances. In those neurons that showed larger Ca^{2+} transients at dendritic locations due to such factors, Ca^{2+} could summate with the paired EPSP to produce a supralinear Ca^{2+} response, and facilitation in EPSC amplitude would result (Camire and Topolnik, 2014). In those neurons that were not able to elicit a strong dendritic Ca^{2+} response, sublinear Ca^{2+} levels would be produced, and a decrease in EPSC amplitude would result.

We also show that the retinogeniculate synapse does not display plasticity with tonic or burst discharge paired with a single EPSP. These findings demonstrate the ability of the sensory pathway to maintain the transfer of virtually unaltered sensory information, from the periphery to the thalamus. These findings are consistent with those of Hsu et al. (2010), who showed that the medial lemniscal pathway (sensory afferents to VB nucleus) did not display plasticity (Hsu et al., 2010). However, in our study unpaired tetanic stimulation of the optic tract produced a lasting depression in retinogeniculate transmission**.** These findings could demonstrate a frequency-dependent effect of the sensory circuit; high-frequency activation of the primary sensory afferents could produce a down regulation in information transfer to the thalamus to compensate for increased sensory stimulation, while at lower frequencies, information transfer to the thalamus is not altered.

In the attempt to enhance the magnitude of state-dependent plasticity at corticothalamic synapses, tonic or burst discharge was paired with tetanic activation of corticothalamic afferents. These results showed state-independent effects, with both paired

stimulation protocols inducing short-term potentiation at corticothalamic synapses. Introduction of tetanic stimulation ameliorated the depression observed following paired tonic stimulation, and produced short-term facilitation irrespective of postsynaptic firing mode**.** We also showed that the unpaired train protocol could induce facilitation without pairing, although the magnitude and duration of facilitation were lower. These data show that the majority of potentiation observed with paired tetanic stimulation can be attributed to the effects of tetanic stimulation. The rationale for the similar potentiation seen with both paired protocols could be attributed to repetitive presynaptic activation, which produces a larger, summated synaptic response. When tetanic stimulation is paired with either firing mode, this could sufficiently elicit a supralinear level of postsynaptic dendritic Ca^{2+}, and cause lasting enhancement following either paired stimulation protocol. It is possible that Ca^{2+} levels are high enough to activate other downstream signaling pathways to produce the long lasting potentiation in both groups where burst and tonic firing is paired with tetanic stimulation.

Lastly, we observed differences at corticoreticular synapses, which displayed long lasting depression following paired burst stimulation**.** These findings are interesting, since the same stimulation paradigm showed both potentiation and depression of EPSC amplitude in relay neurons. These results could reveal more about the way in which the corticothalamic afferents modulate TRN activity on relay neurons. Depressing the synaptic activity of TRN neurons will decrease the inhibitory drive onto relay neurons. Simultaneously, increased cortical feedback will enhance excitation to relay neurons, which together, will act to increase thalamic throughput to the cortex.

This study has shown state-dependent plasticity in thalamocortical relay neurons, and the transition to state-independent potentiation following tetanic stimulation. We have also demonstrated how the TRN primarily produces depression following paired stimulation, which could act together to increase sensory information transfer to the cortex. Additionally, retinogeniculate synapses do not display plasticity, but can suppress synaptic efficacy following high frequency stimulation. These findings could have major implications on the nature of corticothalamic modulation and how information can be altered depending on the state of thalamic neurons and the degree of corticothalamic drive.

APPENDIX

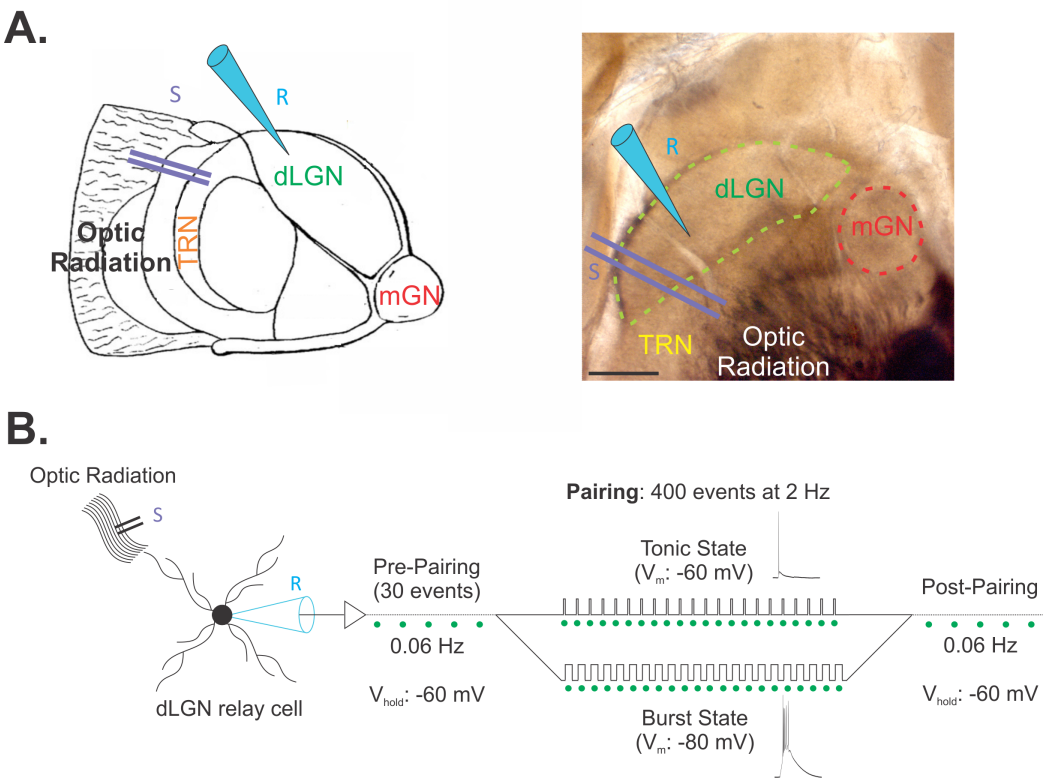

Figure 2.1. Brain slice and induction paradigm used to test state-dependent synaptic plasticity at corticothalamic synapses

A. *Left,* Schematic of parasagittal thalamic slice used, indicating placement of recording (R) and stimulating (S) electrode. *Right,* Image of the parasagittal slice. Scale bar = 550 µm. **B.** Paired stimulation paradigm: EPSCs were evoked at 15-second intervals (0.06 Hz) and at least 30 consecutive, stable responses were recorded (green dots) prior to the paired induction protocol. Prior to initiation of the induction protocols, the recording was switched from voltage clamp mode to current clamp mode, and one of two paired protocols were applied to a given cell. For *paired tonic* stimulation, V_m was kept at -60 mV, and a single action potential was induced by somatic current injection (1000 pA, 2 ms) followed by stimulation of corticothalamic afferents (5 ms latency). For *paired burst* stimulation, V_m was adjusted to -80 mV, and burst discharge was produced by somatic current injection (350 pA, 35 ms) followed by stimulation of corticothalamic afferents (5 ms latency). The paired protocol was repeated 400 times at 2 Hz. Following the paired induction protocol, recordings were switched back to voltage clamp mode and EPSCs were evoked at 15-second intervals

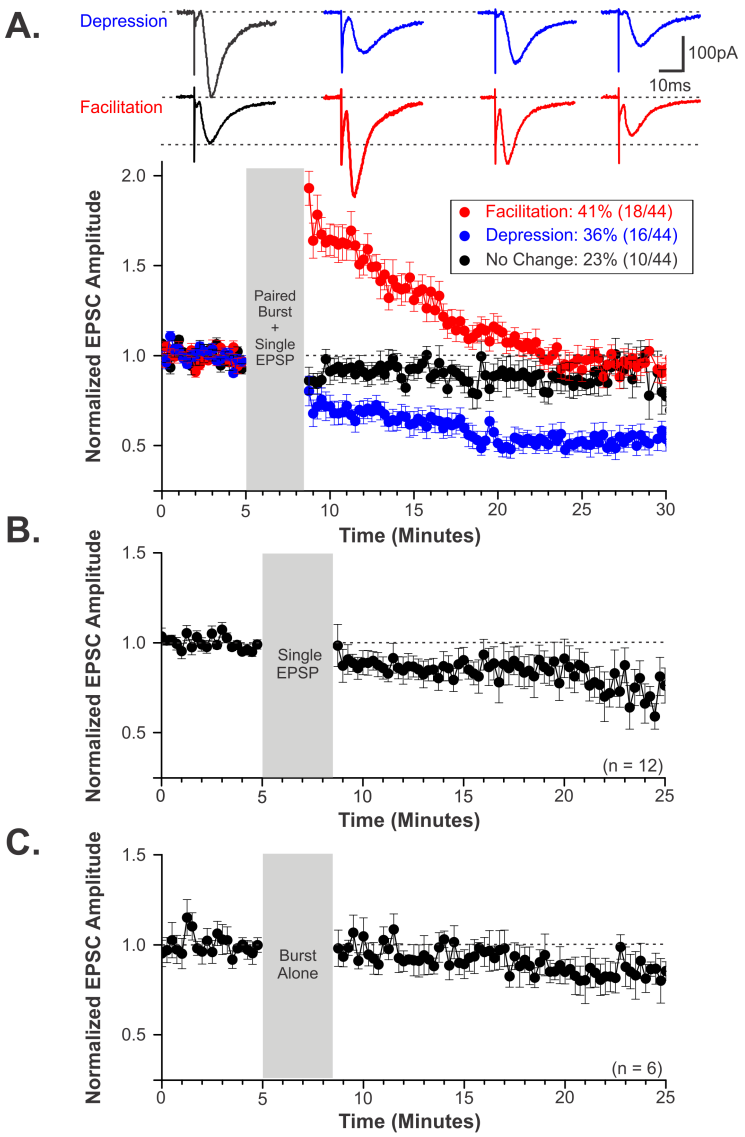

Figure 2.2. Paired burst discharge with a corticothalamic EPSP produces alteration in synaptic responses

A. Paired burst stimulation produced either short-term facilitation or depression of the EPSC. *Top*, Representative EPSCs (average of 5 consecutive responses) before paired burst stimulation (black) and at three different time points after stimulation. The blue traces are from a cell that showed synaptic depression, and the red traces from a cell showing synaptic facilitation, following paired burst stimulation. Graph of population data indicating that 41% of cells (18 of 44) displayed short-term facilitation, 36% of cells (16 of 44) showed synaptic depression, and 23% of cells (10 of 44) displayed unaltered synaptic activity following paired stimulation. **B.** Unpaired single corticothalamic EPSPs alone are not sufficient to produce plasticity. Single corticothalamic EPSPs (400 repetitions at 2 Hz) did not produce alteration in EPSC amplitude (n = 12). **C.** Unpaired bursts alone are not sufficient to produce plasticity. Single bursts (400 repetitions at 2 Hz) did not alter EPSC amplitude (n = 6).

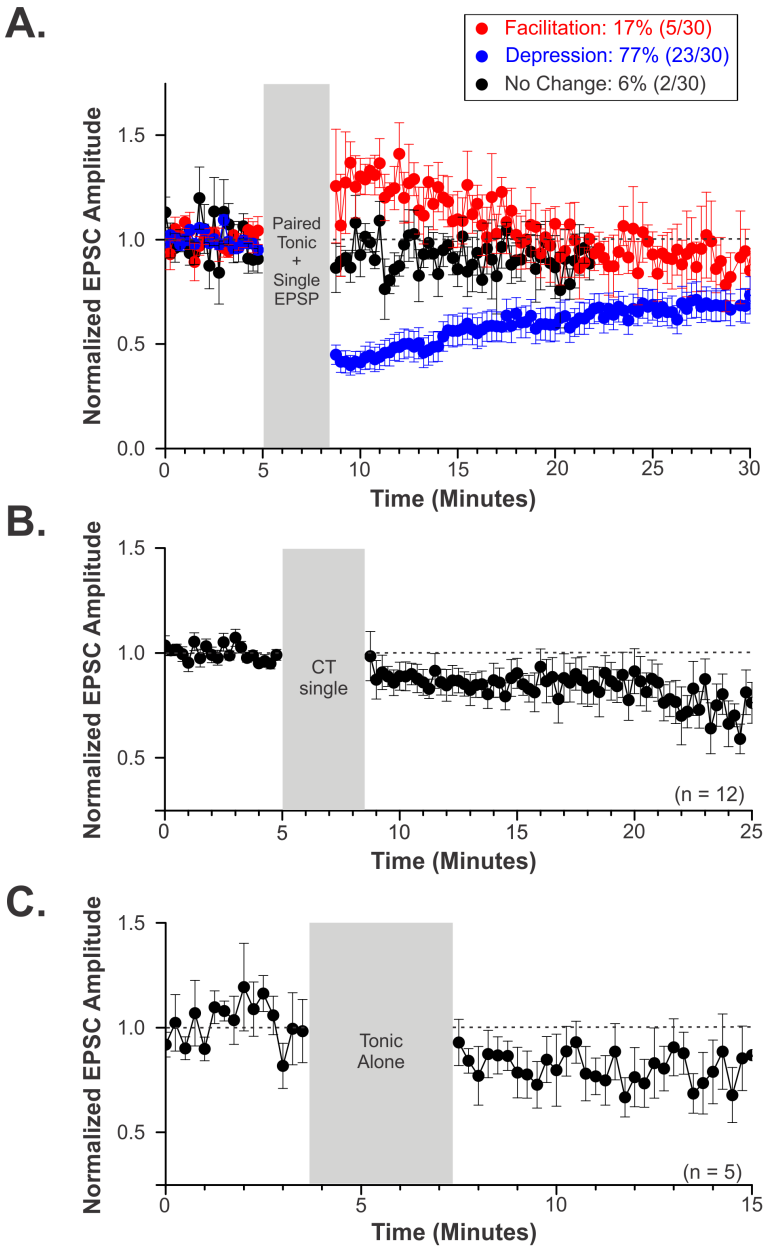

Figure 2.3. Paired tonic firing with a corticothalamic EPSP produces alteration in synaptic responses

A. Paired tonic stimulation produced short-term facilitation and depression of EPSC amplitude. Graph of population data indicating that facilitation of EPSC amplitude was observed in 17% of cells (5 of 30), 77% (23 of 30) showed synaptic depression, and 6% (2 of 30) show unaltered EPSC amplitude following paired tonic stimulation. **B.** Unpaired single corticothalamic EPSPs (400 repetitions at 2 Hz) are not sufficient to produce plasticity (n = 12). **C.** Unpaired single action potentials are not sufficient to produce plasticity. Single action potentials (400 repetitions at 2 Hz) did not alter EPSC amplitude (n = 5).

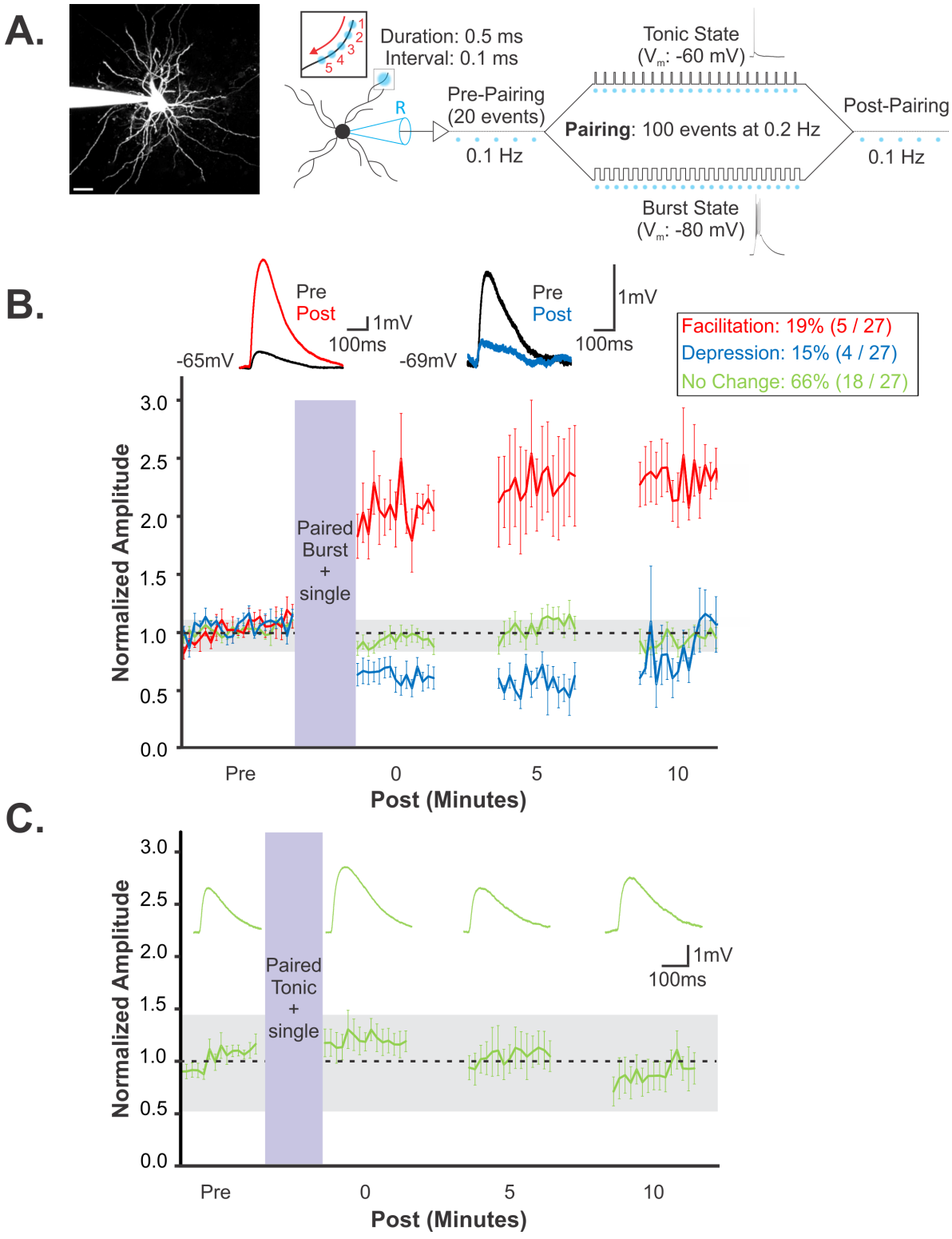

Figure 2.4. Paired burst with glutamate-induced depolarization produced altered amplitudes of responses

Figure 2.4. (cont'd)

A. Paired stimulation paradigm using glutamate uncaging. *Left*, Image of dLGN relay neuron filled with Alexa Fluor 594 (25 μM) Scale bar = 20 μM. Postsynaptic responses were evoked by brief laser stimulation (0.5ms duration, 0.1 ms interval, 5 pulses; 405 nm) over a small dendritic region at distal dendrites (>150 μM from the soma). *Right*, Paired stimulation paradigm: Glutamate depolarizations were collected at 0.1 Hz prior to the induction protocol. For *paired tonic* induction, V_m was adjusted to -60 mV, and a single action potential was induced by somatic current injection (1000 pA, 2 ms), which was closely followed by glutamate uncaging (5 ms latency). For *paired burst* stimulation, V_m was adjusted to -80 mV, and burst discharge was induced by somatic current injection (350 pA, 35 ms), and closely followed by (5 ms latency) glutamate uncaging. The paired stimulation protocol was run for 100 repetitions at 0.2 Hz, and following the paired induction protocol, cell was returned to resting membrane potential and glutamate depolarizations were collected at 10-second intervals **B.** Paired burst stimulation altered postsynaptic responses in 1/3 of cells tested. *Top*, Representative traces (average of 5 consecutive responses) of glutamate-induced depolarizations (Black traces: pre induction; colored traces: post induction). *Bottom*, Graph of population data indicating that 19% of cells (5 of 27) showed facilitation, 15% of cells (4 of 27) showed depression, and the remaining 66% of cells (18 of 27) showed unaltered responses following paired burst stimulation. The 95% confidence interval of baseline variation is represented by the gray box. **C.** Paired tonic stimulation does not produce any alteration in amplitude of glutamate depolarization.

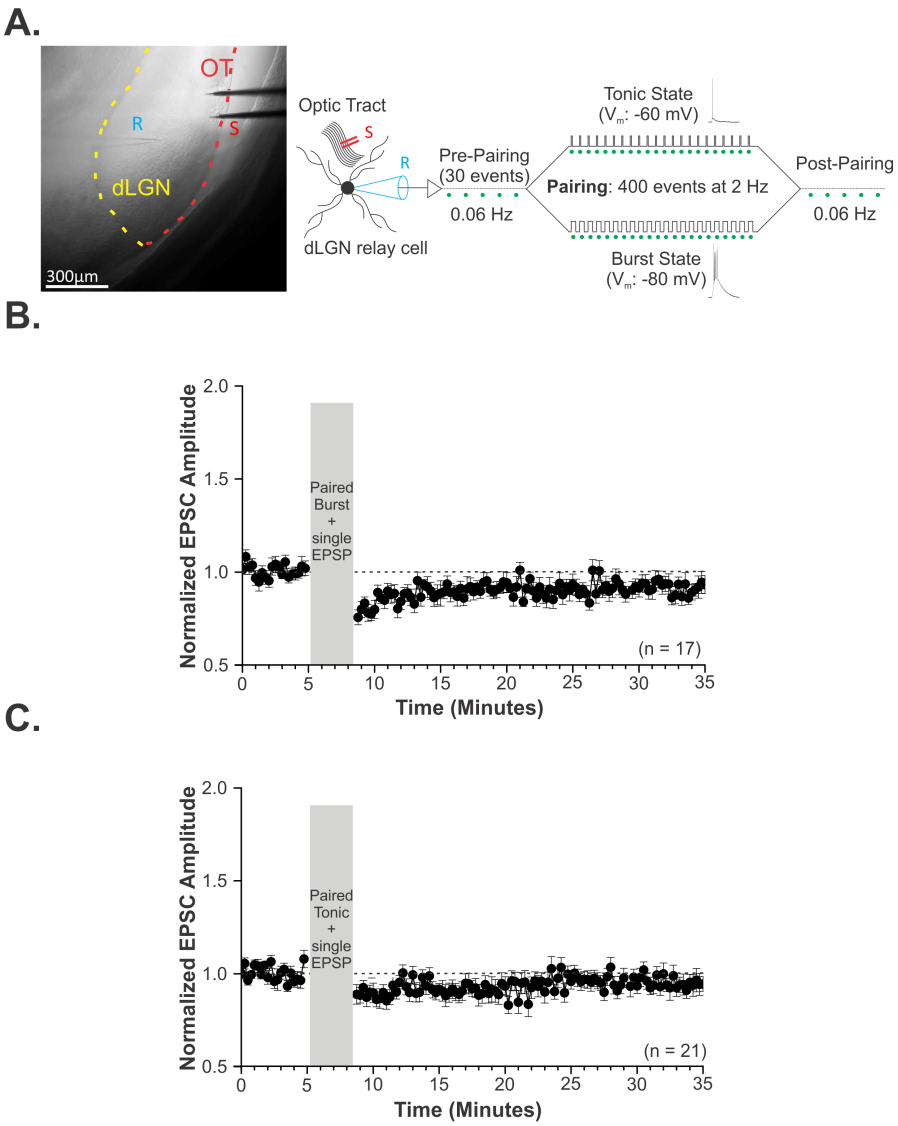

Figure 2.5. Retinogeniculate synapses do not display plasticity following paired stimulation

A. Paired stimulation paradigm at retinogeniculate synapses. *Left*, Image of a coronal thalamic slice used, with placement of recording (R) and stimulating (S) electrode in optic tract (OT). Scale bar = 300 µm. *Right*, Paired stimulation paradigm: EPSPs were evoked at 15-second intervals (0.06 Hz) and 30 consecutive, stable responses were recorded (green dots) prior to the paired induction protocol. For *paired tonic* stimulation, V_m was adjusted to -60mV, and a single action potential was induced by somatic current injection (1000 pA, 2 ms) followed by stimulation of retinogeniculate afferents (latency: 5 ms). For *paired burst stimulation*, V_m was adjusted to -80 mV, and burst discharge was induced by somatic current injection (350 pA, 35 ms), followed by (5 ms latency) stimulation of retinogeniculate afferents to evoke an EPSP. The paired protocol was repeated 400 times at 2 Hz. Following the paired induction protocol, EPSPs were evoked at 15-second intervals. **B.** Paired burst stimulation does not produce any alteration in EPSP amplitude (n = 17). **C.** Paired tonic stimulation does not produce any alteration in EPSP amplitude (n = 21).

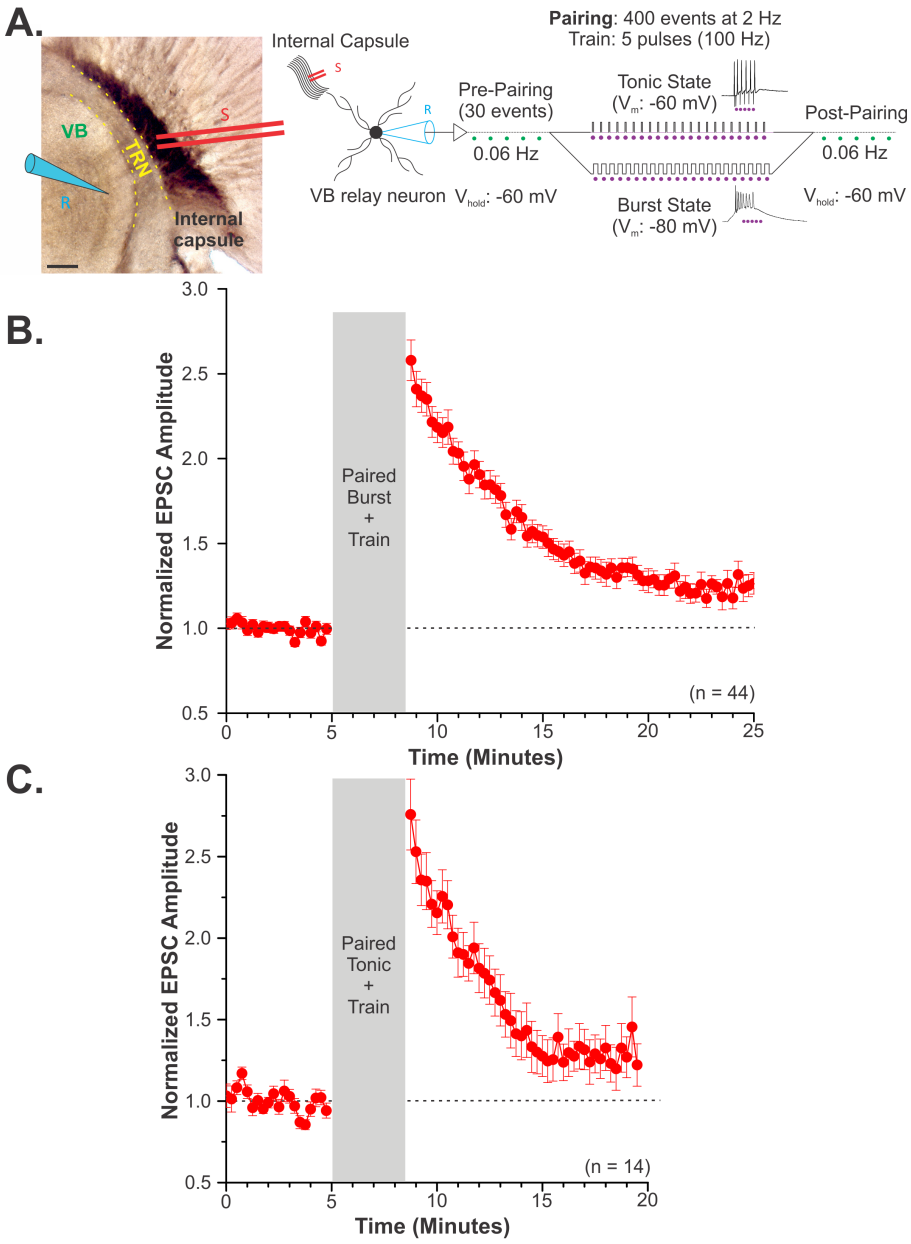

Figure 2.6. Paired stimulation with tetanic activation of corticothalamic afferents produces short-term facilitation

A. Paired stimulation paradigm at corticothalamic synapses in VB relay neurons. *Right*, Image of the horizontal thalamic slice used, with placement of recording (R) and stimulating (S) electrode. Scale bar = 500 µm. *Left*, Paired stimulation paradigm: EPSCs were evoked at 15-second intervals (0.06 Hz) and 30 consecutive, stable responses were recorded (green dots). Prior to initiation of the induction protocols, the recording was switched from voltage clamp mode to current clamp mode, and one of two paired protocols were applied to a given cell. For *paired tonic* stimulation, V_m was adjusted to -60 mV, and a single action potential was induced by somatic current injection (1000 pA, 2 ms) followed by (5 ms latency) tetanic stimulation (5 pulses, 100 Hz, purple dots) of corticothalamic afferents. For *paired burst* stimulation, V_m was adjusted to -80 mV, and burst discharge was

Figure 2.6. (cont'd)

induced by somatic current injection (350 pA, 35 ms) followed by (5 ms latency) tetanic stimulation (5 pulses, 100 Hz, purple dots) of corticothalamic afferents. The paired protocol was repeated 400 times at 2 Hz. Following the paired induction protocol, recordings were switched back to voltage clamp mode and EPSCs were evoked at 15-second intervals. **B.** Paired burst + train produces short-term facilitation. Graph of population data indicating that 94% of cells (44 of 47) displayed short-term facilitation, and 6% of cells displayed unaltered EPSC amplitude (data not shown). **C.** Paired tonic + train produces short-term facilitation. Graph of population data indicating that 93% (14 of 15) of cells displayed facilitation, and 7% of cells showed unaltered EPSC amplitude (data not shown).

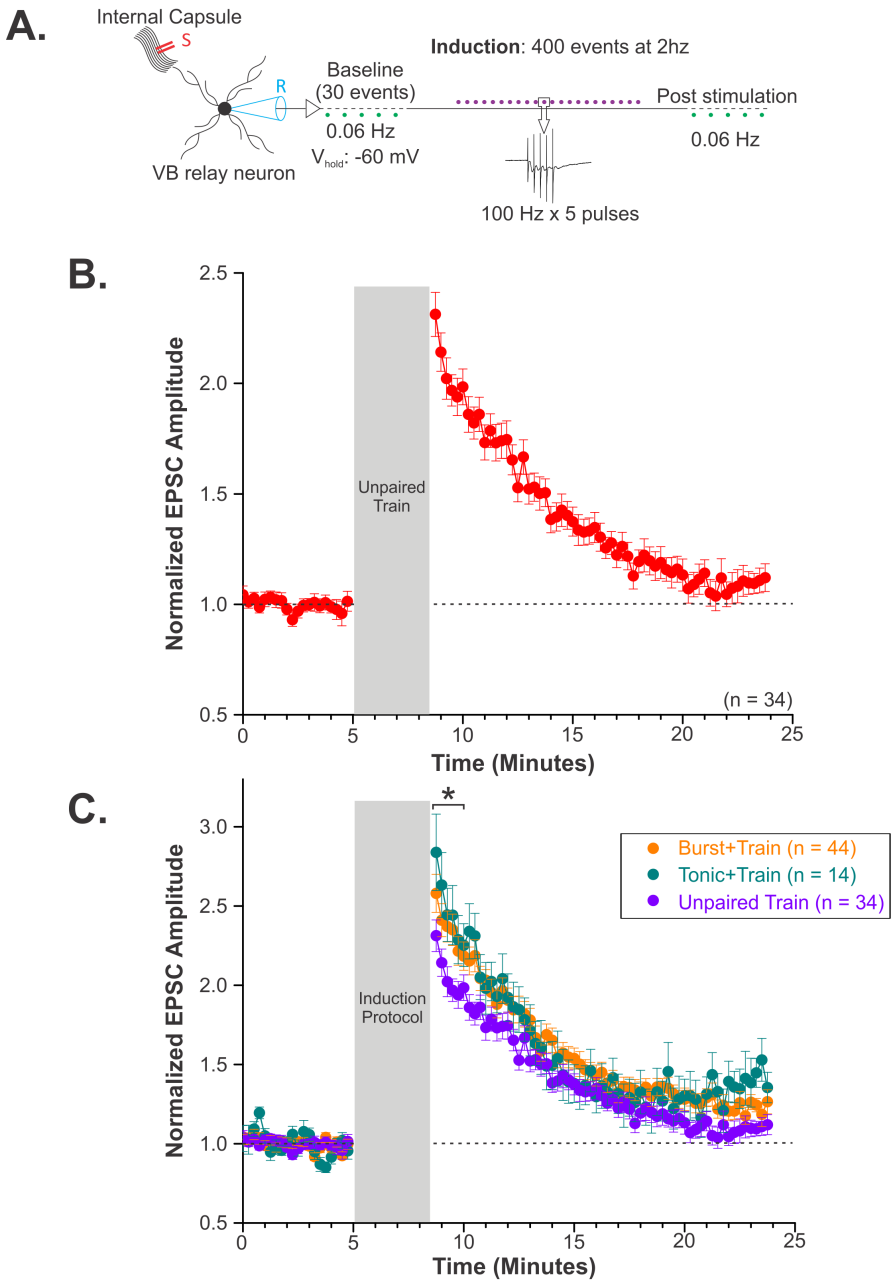

Figure 2.7. Unpaired tetanic stimulation of corticothalamic afferents produces short-term facilitation

A. Unpaired stimulation paradigm: corticothalamic EPSCs were at 15-second intervals (0.06 Hz) and 30 consecutive, stable responses were recorded (green dots) prior to the induction protocol. Induction involved tetanic stimulation (5 pulses at 100 Hz), repeated 400 times at 2 Hz (purple dots). Following induction, EPSCs were evoked at 15-second intervals **B.** Unpaired stimulation produces short-term facilitation. The graph of population data shows 81% of cells (34 of 42) displayed short-term facilitation in EPSC amplitude, and 19% of cells displayed unaltered EPSC amplitude (data not shown). **C.** Comparison of data for paired tetanic and unpaired tetanic stimulation protocols. Burst and tonic paired with tetanic stimulation did not significantly differ, but unpaired tetanic stimulation displayed lower magnitude of facilitation (p = 0.004).

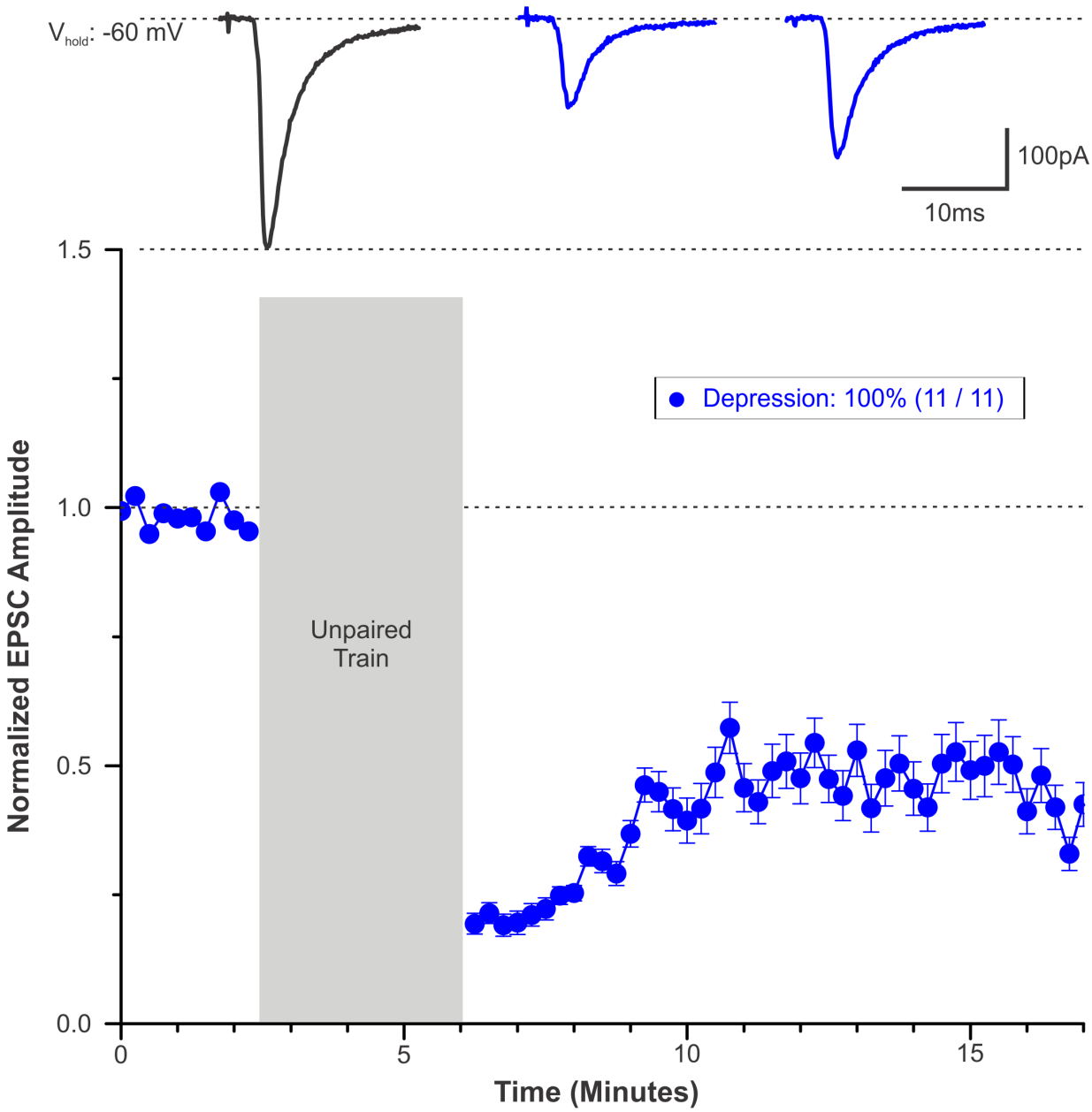

Figure 2.8. Retinogeniculate synapses display depression following unpaired tetanic stimulation

Top, Representative EPSCs (average of 5 consecutive responses) before tetanic stimulation (black) and at three different time points after stimulation (blue). *Bottom*, Graph of population data shows depression of EPSC amplitude following tetanic stimulation of retinogeniculate synapses (n = 11).

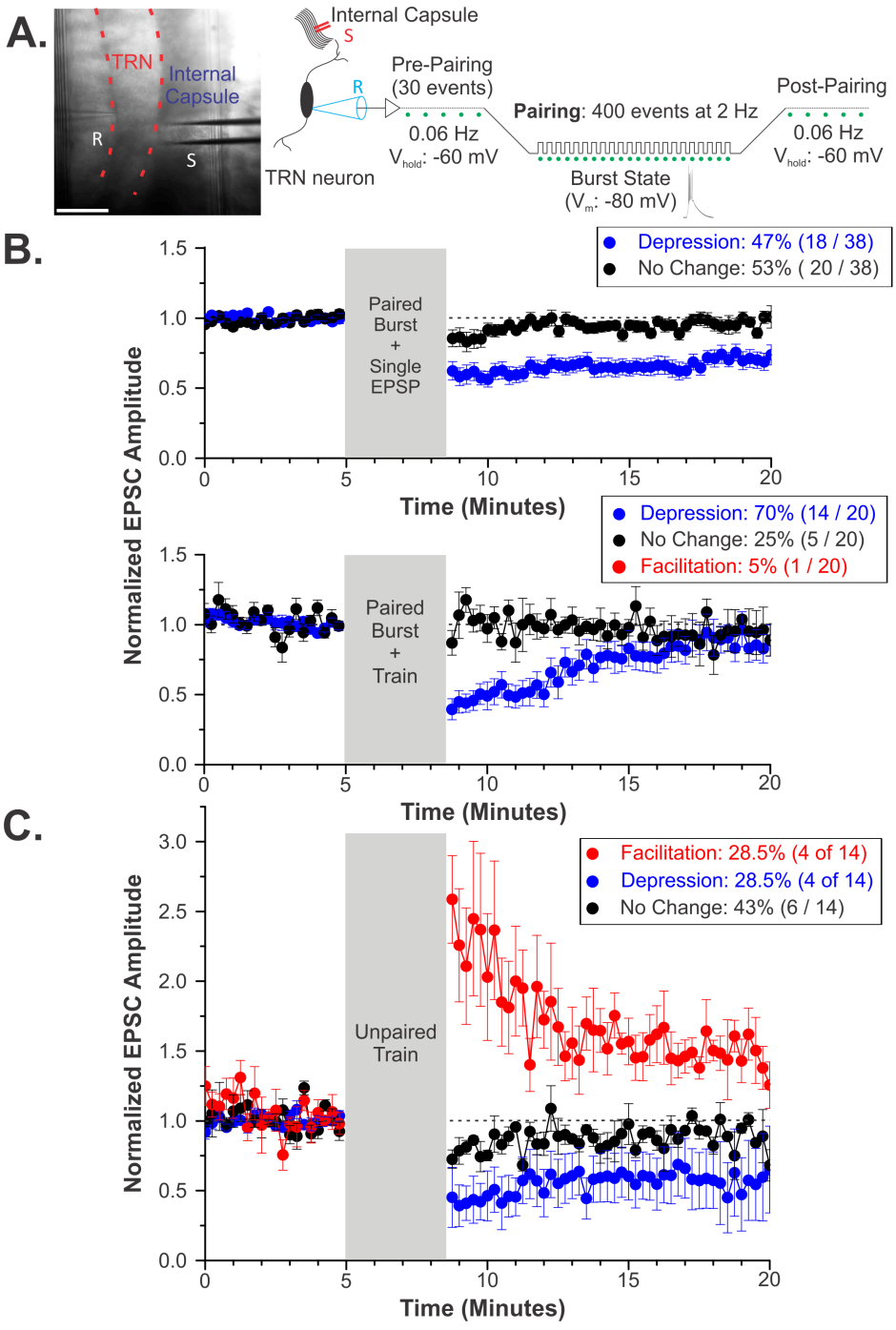

Figure 2.9. Paired burst stimulation with corticothalamic EPSP produces depression in TRN neurons

A. Paired stimulation paradigm in TRN neurons. *Left*, Image of horizontal thalamic slice used, indicating placement of recording (R) and stimulating (S) electrode. Scale bar = 300 µm. *Right*, Paired stimulation paradigm: EPSCs were evoked at 15-second intervals (0.06 Hz) and 30 consecutive, stable responses were recorded (green dots). Prior to initiation of the induction protocols, the recording was switched to current clamp mode, and one of either paired burst protocols were applied to a given cell. For paired burst stimulation, V_m was adjusted to -80 mV, and burst discharge was induced by somatic current injection (350

Figure 2.9. (cont'd)

pA, 35 ms) followed by (5 ms latency) by either single stimulation of corticothalamic afferents to evoke an EPSP, or tetanic stimulation (5 pulses, 100 Hz) of corticothalamic afferents to evoke a train of EPSPs. The paired protocol was repeated 400 times at 2 Hz. Following the paired induction protocol, recordings were switched back to voltage clamp mode and EPSCs were evoked at 15-second intervals. **B.** Paired burst stimulation produces depression in TRN neurons. *Top,* Graph of population data following paired burst + single EPSP, indicating that 47% of cells (18 of 38) displayed depression, and 53% of cells (20 of 38) showed unaltered EPSC amplitude. *Bottom*, Graph of population data following burst + train stimulation, indicating that 70% of cells (14 of 20) displayed depression, and 25% of cells (5 of 20) showed unaltered EPSC amplitude. **C.** Unpaired tetanic stimulation produces alteration in synaptic responses. Graph of population data indicating 28.5% of cells (4 of 14) display facilitation, 28.5% of cells (4 of 14) show depression, and 43% of cells (6 of 14) show unaltered EPSC amplitude following tetanic stimulation.

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CHAPTER III: SHORT-TERM PLASTICITY AT CORTICOTHALAMIC SYNAPSES IN THALAMOCORTICAL NEURONS

3.1 Introduction

Thalamocortical circuits have been associated with many functions, including sensory processing, alterations of behavioral states (i.e. sleep vs. wakefulness, attention, arousal), as well as pathophysiological conditions, such as sensory processing disorders and absence epilepsy (Gloor P., 1984; Steriade, 1993; Steriade and Contreras, 1995; von Krosigk et al., 1999; Bereshpolova et al., 2011). It is clear that information transfer at subcortical levels is not just a passive relay of sensory information as traditionally thought, but involves dynamic processing (Jones, 1985; Dinse et al., 1997; Sherman and Guillery, 2002; Crandall et al., 2010; Tyll et al., 2011; Govindaiah et al., 2012).

The ventrobasal nucleus (VB) is the primary somatosensory nucleus of the thalamus. Thalamocortical relay neurons in VB receive sensory inputs from the medial lemniscal pathway and project to the primary somatosensory region of the cerebral cortex (Jones, 1985; Liu et al., 1995). Primary sensory afferents, however, only account for ~10% of synaptic contacts onto thalamic relay neurons (Liu et al., 1995).

Corticothalamic feedback projections provide a large number of afferents to the thalamus (Guillery, 1969; Liu et al., 1995; Erisir et al., 1997) ((Gilbert and Kelly, 1975; Dreher and Sefton, 1979; Jones, 1985); therefore, corticothalamic feedback could play a significant role in the modulation of information transfer, and thus serve as a critical player in dynamic processing at the subcortical level.

Corticothalamic feedback has been shown to regulate relay neuron excitability (Deschenes and Hu, 1990; Godwin et al., 1996), facilitate cortico-cortical communication

with the thalamus as an intermediary (Guillery and Sherman, 2002), and affect sensory receptive field organization (Yuan et al., 1985; Ergenzinger et al., 1998), demonstrating its ability to exert control over sensory information processing.

Synaptic plasticity in the thalamocortical circuit has been of particular interest in recent studies (Lindstrom and Wrobel, 1990; Ergenzinger et al., 1998; Castro-Alamancos and Calcagnotto, 1999; Krupa et al., 1999; Connelly et al., 2016), due to the role of corticothalamic feedback afferents in dynamically impacting thalamic throughput and perception. Alterations in synaptic efficacy in the thalamocortical circuit could be one mechanism by which the cortex can influence information flow through the circuit, which can potentially enhance transmission of salient information.

Several studies have addressed the implications of long-term plasticity in thalamocortical circuits (Castro-Alamancos and Calcagnotto, 1999; Hsu et al., 2010, 2012; Astori and Luthi, 2013; Sieber et al., 2013). Long-term potentiation, or the lasting enhancement of excitatory synaptic efficacy, in these cases, can impact communication between the cortex and thalamus for prolonged periods of time. By increasing excitation, the cortex could effectively influence the gain of information flow through the thalamocortical circuit. While increasing thalamocortical communication could enhance information transfer, the behavioral implications of long-term alterations in this pathway are still not well understood. In the sensory circuit, corticothalamic communication may require more dynamic regulation depending on changing environmental stimuli. Therefore, short-lasting changes in synaptic efficacy could be a relevant theory for corticothalamic regulation of communication within this pathway.

It has been previously shown that short-term plasticity, lasting on the order of milliseconds to minutes, at synapses in the thalamocortical circuit, can lead to receptive field reorganization (Nicolelis et al., 1993; Dinse et al., 1997; Ergenzinger et al., 1998; Krupa et al., 1999). Short-term alterations of corticothalamic synaptic efficacy (i.e. enhancement or suppression of neurotransmission) may represent changes at the synaptic level, which are not due to anatomical or transcriptional changes. These transient changes can contribute to more dynamic regulation of cortical control, since they can be modulated by sensory input or behavior state. For example, in response to a particular sensory stimulus, increased corticothalamic activation of relay neurons can enhance the flow of sensory information through the circuit, thereby increasing the relay neuron's communication with the cortex, and enhancing transmission of more salient information. A mechanism to alter synaptic efficacy and thereby modulate information transfer to the cortex in a behavior-relevant manner could be one form of subcortical dynamic information processing within thalamocortical circuits.

In this study, we found that tetanic stimulation of corticothalamic afferents produced short-term facilitation of excitatory synaptic currents in thalamocortical relay neurons, which lasts for approximately 15-20 minutes. We show that short-term facilitation is a result of a presynaptic mechanism, which stimulates intracellular signaling pathways, leading to altered ion channel conductance. We hypothesize that these changes contribute to enhanced neurotransmitter release. These results provide a detailed mechanism through which high-frequency corticothalamic activity could potentially regulate information processing through thalamocortical circuits.

3.2 Materials and Methods

All experimental procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Michigan State University Institutional Animal Care and Use Committee. We used the minimal number of animals necessary to complete these experiments.

Thalamic brain slices were prepared from Sprague Dawley rats (postnatal age: 15-25 days) of either sex. Animals were deeply anesthetized with isofluorane (2-4% inhalation), perfused with cold (4°C), oxygenated (5% CO_2, 95% O_2) slicing solution, and quickly decapitated. Brains were removed and placed in slicing solution containing the following (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgSO_4$, 0.5 $CaCl_2$, 26.0 $NaHCO_3$, 10.0 glucose, and 234.0 sucrose. Thalamic slices (300-400 μm) were cut in the horizontal plane for VB recordings using a vibrating tissue slicer. Slices were immediately transferred to a holding chamber with oxygenated physiological saline containing the following (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose. After 20-30 minutes, the holding chamber was reduced from 35°C to room temperature (~25°C), and slices were incubated for an additional 60 minutes before recording.

For electrophysiological recordings, individual slices were transferred to a recording chamber that was maintained at 30°C with oxygenated physiological saline (2.5-3 ml/min). Individual relay neurons were identified using a microscope equipped with differential interference contrast optics and 63x water-immersion objective (Zeiss Axioexaminer). Recording pipettes were made from 1.5mm OD borosilicate glass and had a tip resistance of 2-6 MΩ when filled with the following internal solution (in mM): 117 K-gluconate, 13.0 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2-ATP, and 0.4 Na-

GTP, pH 7.3 (290 mOsm). The internal solution resulted in a junction potential of ~10 mV and was corrected for in all voltage recordings. During recordings, pipette capacitance was neutralized, and access resistance was continually monitored. Recordings had access resistances <20MΩ. In addition, input resistance and holding current were continually monitored throughout the experiment.

Recordings were made using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Voltage-clamp recordings were collected at a holding potential of -60 mV. Excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulation (duration: 100 μs, intensity: 50-500 μA) of corticothalamic afferents by placing a bipolar tungsten electrode in the internal capsule or in the thalamic reticular nucleus. In order to isolate EPSCs, all experiments were done in the presence of $GABA_A$ and $GABA_B$ antagonists, 6-Imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid hydrobromide (SR-95531 hydrobromide; 10μM) and *N*-[[*trans*-4-[[(4-Amino-2-quinazolinyl)amino]methyl]cyclohexyl]methyl]-1-naphthalenesulfonamide hydrochloride (CGP-54626 hydrochloride; 5μM) respectively.

Antagonists and agonists were prepared and stored as recommended and diluted in physiological saline to a final concentration before use. All antagonists were bath applied for 5-10 min before subsequent experimental tests. Agonists were applied by bolus injection into the flow line of the chamber using a motorized syringe pump. Based on the rate of agonist injection and chamber perfusion, the final bath concentration of agonists were estimated to be approximately one-fourth of the concentration introduced into the flow line (Cox and Sherman, 1999). All compounds were purchased from Tocris, Sigma-Aldrich, or Santa Cruz Biotechnology.

A. Stimulation paradigm

Baseline corticothalamic synaptic activity was initially obtained by electrically stimulating the internal capsule or TRN, while recording from VB relay neurons (**Fig. 3.1A**). Evoked corticothalamic EPSCs were pharmacologically isolated with $GABA_A$ and $GABA_B$ antagonists to eliminate any inhibitory effects from the TRN. EPSC amplitudes were recorded in voltage-clamp mode (V_{hold}=-60mV) at 15-second intervals, and 30 consecutive, stable responses were collected for baseline levels of excitatory synaptic activity. Next, the tetanic stimulation protocol was applied. The stimulation protocol consisted of a corticothalamic train (5 pulses at 100 Hz), repeated 400 times at 2 Hz (**Fig. 3.1B**). Following tetanic stimulation, EPSCs were evoked (15-second intervals) and peak amplitude was compared to that of baseline levels.

Data acquisition and analyses were performed using pCLAMP software (Molecular Devices). Data were digitized at 10 kHz, filtered online at 2.5 kHz, and stored on computer for posthoc analyses. Pre- and post-induction EPSC amplitudes were compared, and cells were classified into groups that displayed either facilitation or unaltered synaptic responsiveness. Facilitation was defined by a 2x standard deviation (SD) increase in EPSC amplitude above pre-induction amplitudes that persisted for at least 10 consecutive responses (2.5 min). Responses were compiled and normalized to 1 to form the population data.

Magnitude changes of EPSC amplitude from baseline (10 consecutive responses prior to induction protocol) were compared to those following the stimulation protocol (first 5 responses post induction) or at the peak drug effect (5 consecutive responses). Comparisons based on magnitude were made using a Student's *t* test. Mixed-design ANOVA

models were used to examine mean differences in control groups and those with pharmacological manipulations, across time (initial 5 minutes following stimulation or first four minutes of agonist application). The within-group variables were time (minutes), while the between subjects factor was control versus drug. Data are presented as Mean ± SEM, and statistical significance was assessed with p value < 0.05.

3.3 Results

For these experiments, whole-cell recordings were obtained from 331 thalamocortical relay neurons.

A. Thalamocortical relay neurons display short-term facilitation following tetanic stimulation

Following collection of stable EPSC amplitude, tetanic stimulation of corticothalamic afferents produced a facilitation of EPSC amplitude in thalamocortical neurons with a duration of several minutes (**Fig. 3.2A**). The facilitation was observed in 34 of 42 cells (81%) tested. Overall, in cells that show short-term facilitation, the magnitude of facilitation peaked at a 108.6 ± 7.7% increase from baseline, and typically recovered within 10-15 minutes (**Fig. 3.2B**).

B. Short-term facilitation is not mediated by mGluR activation

To determine the mechanism of short-term facilitation, we first tested the possible contribution of metabotropic glutamate receptor (mGluR) activation in mediating our effects, because high-frequency tetanic stimulation of corticothalamic afferents can activate mGluRs in thalamocortical neurons (von Krosigk et al., 1999). If the facilitation was dependent on mGluR activation, we hypothesized that an mGluR agonist should mimic the facilitation of the EPSC. The general mGluR agonist, (1S,3R)-ACPD (ACPD), was

administered by brief bath application (50μM; 225 s), and produced a strong suppression of EPSC amplitude (60 ± 3.7%, n = 12; data not shown). While the suppression of EPSC amplitude is opposite to the facilitation, activation of mGluR subtypes (i.e. group I and II) have been shown to have opposite actions on synaptic activity or membrane potential of thalamic neurons (Cox and Sherman, 1999; Mateo and Porter, 2007), and thus we tested selective Group I and Group II mGluR agonists in case there was a similar effect on EPSC amplitudes. The group I agonist, (RS)-3,5-DHPG (DHPG; 50μM) produced a similar suppression of the EPSC amplitude as with ACPD (76.4 ± 3.4%, n = 5; data not shown). The group II agonist, (2R,4R)-APDC (APDC; 75 μM) also suppressed EPSC amplitude by 38 ± 1.5% (n = 3). These data indicate that mGluR activation is not contributing to tetanus-induced facilitation.

C. Short-term plasticity is mediated by presynaptic mechanisms

In the next series of experiments, we sought to determine if tetanus-induced facilitation is mediated by pre- and/or postsynaptic mechanisms. Evoked EPSCs consist of ionotropic glutamatergic currents from activation of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and N-methyl-D-aspartate receptors (NMDAR). If short-term facilitation is due to presynaptic mechanisms, then we predict that both NMDAR- and AMPAR-mediated synaptic currents would be facilitated, whereas differential facilitation of the isolated responses would be consistent with a postsynaptic site of action. Therefore, we pharmacologically isolated the AMPAR- or NMDAR-mediated EPSCs, and tested if these individual components were altered by the induction protocol.

The NMDAR-mediated EPSCs were evoked at a holding potential of -25 to -40 mV in the presence of AMPAR antagonist DNQX (20μM). After obtaining a stable EPSC amplitude,

the tetanic stimulation protocol was applied and the NMDAR-mediated EPSC was strongly facilitated (218.7 ± 44.1%, n = 7; **Fig. 3.3A**) . AMPAR-mediated EPSCs were evoked at a holding potential of -60 mV in the presence of the NMDAR antagonist RS-CPP (CPP; 10μM). Similarly, tetanic stimulation also produced strong short-term facilitation of AMPAR-mediated EPSCs (158.5 ± 27.8%, n = 9; **Fig. 3.3B**). These results lead us to two conclusions. First, this form of plasticity is not dependent on NDMAR activation, as is the case with many other forms of synaptic plasticity in other brain regions (Bliss and Collingridge, 1993; Malenka and Nicoll, 1993). Second, since both NMDAR- and AMPAR-dependent synaptic currents displayed potentiation, these data are consistent with a presynaptic site of action for short-term facilitation.

Next, we looked at alterations in paired-pulse ratio, considering this may serve as an additional measure for distinguishing between the pre- and post-synaptic site of action. Paired-pulse stimulation of the corticothalamic pathway (100 ms interstimulus interval) produced facilitation of the second EPSC ($EPSC_2$) relative to first EPSC ($EPSC_1$) (**Fig. 3.4A**, inset) similar to that previously reported (Turner and Salt, 1998; von Krosigk et al., 1999). Following tetanic stimulation there was robust facilitation of $EPSC_1$ amplitude; however, $EPSC_2$ amplitude was unaltered (**Fig. 3.4A,** n = 27). As a result of this, the paired-pulse ratio ($EPCS_2/EPSC_1$) was significantly decreased following tetanic stimulation (**Fig. 3.4B**; p = 0.018; paired t test). Observing that $EPSC_2$ amplitude is unaltered following tetanic stimulation, we ensured that the $EPSC_2$ response was not at a maximum amplitude. Before baseline collection, we determined the maximum amplitude of the EPSC, and then adjusted the stimulus current to evoke $EPSC_2$ amplitude at approximately 50% of maximum. The

decrease in paired-pulse ratio following tetanic stimulation further supports the hypothesis that short-term facilitation is occurring through presynaptic mechanisms.

D. Retrograde messengers do not contribute to short-term facilitation

Considering short-term facilitation may be due to a presynaptic mechanism, it could be the case that a retrograde signal may be involved. Retrograde signaling has been shown to affect neurotransmitter release in multiple thalamic nuclei (Usunoff et al., 1999; Alexander et al., 2006; Yang and Cox, 2007; Sun et al., 2011), so we first tested the possible role of nitric oxide (NO). Brief bath application of the NO donor, SNAP (500 μM; 3.75 minutes), produced a robust increase in the EPSC amplitude (107.1 ± 15.1% , n = 5; **Fig. 3.5A**). Since the SNAP-induced potentiation of EPSC amplitude was similar to the tetanus induced facilitation, we next tested if the two actions could be engaging the same pathway. We performed an occlusion experiment in which tetanic stimulation was carried out at the peak of the SNAP-induced facilitation of EPSC amplitude. We predicted that if tetanus-induced facilitation were engaging the same mechanism as NO signaling, then tetanic stimulation in the presence of SNAP would not induce further potentiation of the EPSC. First, SNAP was bath applied, and once the potentiation of the EPSC amplitude stabilized, tetanic stimulation was carried out. Following tetanic stimulation in the presence of SNAP, the EPSC amplitude was facilitated by 255.3 ± 55.2% (n = 3; **Fig. 3.5B**). The large magnitude of increase suggests that there is an additive effect of tetanus-induced facilitation and SNAP-induced facilitation, and that the tetanus-induced facilitation is not engaging the NO pathway. To further confirm this interpretation, we also tested if NO inhibitors could attenuate the tetanus -induced facilitation. In the presence of the NO inhibitor, Carboxy-PTIO (PTIO; 20 μM), tetanic stimulation caused a strong facilitation of

EPSC amplitude, which did not significantly differ from short-term facilitation in control conditions (**Fig. 3.5C**; n = 7; $F_{14,602}$ = 1.36; p = 0.23). We also tested a different inhibitor, ODQ, which blocks the activity of NO-sensitive guanylyl cyclase. In the presence of ODQ (50-100 μM), tetanic stimulation produced a robust facilitation of EPSC amplitude that did not significantly differ from control conditions (**Fig. 3.5D**; n = 3; $F_{11,429}$ = 0.22; p = 0.97). These data indicate that tetanic stimulation does not activate the NO signaling pathway.

Another group of retrograde messengers that have effects on synaptic release in the thalamus are endocannabinoids (Sun et al., 2011). Brief, bath application of the CB_1 /CB_2 receptor agonist, WIN 55,212-2 mesylate, (10 μM) produced a strong suppression of EPSC amplitude (78.7 ± 4.1%, n=3; data not shown). From this, we conclude that activation of endocannabinoid receptors are not responsible for the tetanus-induced facilitation of EPSCs.

E. Short-term facilitation is Ca^{2+}-dependent

Since many forms of synaptic plasticity, including presynaptic mechanisms, commonly involve Ca^{2+}-dependent mechanisms (Eccles, 1983; Zucker, 1999; Rose and Konnerth, 2001; Nelson et al., 2002), the objective of the next set of experiments were to determine if short-term facilitation is Ca^{2+}-dependent. After stable EPSC amplitude was obtained in normal extracellular physiological saline (Methods), the extracellular solution was changed to a Ca^{2+}-free physiological saline (0 mM Ca^{2+}, 4 mM Mg^{2+}). As expected, washing in the Ca^{2+}-free saline attenuated EPSC amplitude (**Fig. 3.6A**). The tetanic stimulation protocol was applied at this time, and following stimulation, control saline was washed in. Within ~3-4 minutes, the EPSC amplitude returned towards baseline levels. A similar observation was obtained in all cells tested (**Fig. 3.6B**; n = 17). It is important to

note that tetanus-induced facilitation was not observed in any of the cells tested. While facilitation was not observed, there suppression of EPSC amplitude following tetanic stimulation (28.0 ± 17.6%; $p < 0.0001$, paired *t* test). To verify that this suppression was not due to effects of washing in the Ca^{2+}-free solution, Ca^{2+}-free control experiments were run (**Fig. 3.6C**). Ca^{2+}-free physiological saline was washed in for the same duration as the stimulation experiments, but tetanic stimulation was not delivered. The Ca^{2+}-free controls also displayed suppression of EPSC amplitude (15.8 ± 16.6%, n = 4; $p = 0.04$, paired *t* test), indicating that the Ca^{2+}-free solution alone also suppresses EPSC amplitude.

Since short-term facilitation was reliably blocked in the absence of Ca^{2+}, we wanted to determine if plasticity could be reestablished in the same cell, considering the Ca^{2+}-free solution produced a lasting suppression of EPSC amplitude, and facilitation was observed in 81% of neurons in control conditions. For these experiments, tetanic stimulation was delivered in the Ca^{2+}-free saline, and again showed no facilitation following wash in of the normal extracellular solution (**Fig. 3.7A**). In the control solution, a new baseline was established, and the subsequent tetanic stimulation protocol produced robust facilitation of EPSC amplitude (124.7 ± 16.9%, n = 6; **Fig. 3.7B**), which did not significantly differ from facilitation in control conditions ($p = 0.94$; unpaired *t* test).

Since the Ca^{2+}-free saline does not distinguish between pre- or postsynaptic site of action, we took advantage of the whole-cell configuration to alter postsynaptic Ca^{2+} levels using Ca^{2+} chelators, BAPTA (10mM) and EGTA (2mM). These two compounds were included in the recording pipette, and after the whole-cell configuration was achieved, the chelators were left to perfuse into the cell for at least 20 minutes. In order to determine if the chelators were effective, we tested whether the low threshold Ca^{2+} current was

attenuated in these conditions. Depolarizing steps in a current-voltage protocol were given, and a reduction in I_T-mediated, low-threshold spike amplitude signified that Ca^{2+} was effectively being chelated from within the cell. Most cells showed a reduction in LTS, to the point that a burst could no longer be induced. Stable EPSC amplitudes were established, and then tetanic stimulation was applied. Following stimulation, the EPSC amplitude was facilitated, and did not significantly differ from control conditions (**Fig. 3.8**, n = 8; $F_{15,720}$ = 1.50, p = 0.10). These data further support the hypothesis that short-term facilitation is due to a presynaptic mechanism.

F. Short-term facilitation is dependent on adenylyl cyclase activation

Now that we have shown that short-term plasticity is presynaptic and Ca^{2+}-dependent, we sought to determine the presynaptic, intracellular signaling pathway that may be involved in initiating tetanus-induced plasticity. Ca^{2+} binding to specific proteins can directly act on intracellular signaling molecules, initiating signaling cascades (Hanoune and Defer, 2001; Ferguson and Storm, 2004; Ehling et al., 2012). The adenylyl cyclase pathway is one example of a ubiquitous intracellular enzyme that initiates signaling cascades, and can be activated by Ca^{2+}. To test the possible role of adenylyl cyclase in intracellular signaling, we determined if the adenylyl cyclase activator, forskolin, could alter evoked EPSC amplitudes. Short bath application of forskolin (10 μM; 3.75 minutes) produced a robust potentiation in EPSC amplitude (104.6 ± 13.9%, n = 6; **Fig. 3.9A**), which typically recovered within 15 minutes.

Next, we determined if the tetanus-induced facilitation is dependent on adenylyl cyclase activation by conducting an occlusion experiment in which tetanic stimulation was applied in the presence of forskolin. Once the forskolin-induced potentiation of the EPSC

stabilized, tetanic stimulation was applied, and all cells failed to show facilitation of EPSC amplitude (**Fig. 3.9B**; n = 11). Some cells showed suppression following stimulation (10.5 ± 9.1%; $p = 0.02$, paired t test). Since facilitation was completely inhibited in the presence of forskolin, this indicates that the facilitation occurs through the adenylyl cyclase signaling pathway.

Since the occlusion experiments provided evidence that short-term facilitation is adenylyl cyclase-dependent, we next tested the adenylyl cyclase inhibitor, SQ-22536 , to determine if it could attenuate the tetanus-induced facilitation. In order to determine an effective concentration of SQ-22536, we tested if the antagonist would attenuate the forskolin-induced potentiation. In the presence of SQ-22536 (50 μM), application of forskolin (10 μM, 3.75 minutes) produced a smaller potentiation of EPSC amplitude (47.2 ± 10%; n = 11), which was significantly reduced compared to the control response (**Fig. 3.9C**; $F_{32,448} = 12.45$, $p < 0.0001$). We next tested if SQ-22536 would alter the tetanus-induced facilitation of the EPSC. In the presence of SQ-22536 (50 μM), we show that tetanus-induced facilitation of the EPSC was significantly attenuated compared to control conditions (**Fig. 3.9D**, n=22; $F_{17,986}=1.86$, $p = 0.018$).

To further elucidate the pre- versus post-synaptic site of action for adenylyl cyclase activity, the cAMP analogue, 8-Bromo-cAMP, was included in the recording pipette to occlude cAMP activity in the postsynaptic cell. If facilitation does not occur following tetanic stimulation, as was seen in the forskolin occlusion experiments, this is indicative of a postsynaptic site of adenylyl cyclase activation and cAMP production. After the whole-cell configuration was achieved, 8-bromo-cAMP (10mM) was left to perfuse into the cell for ~20 minutes. Once stable EPSC amplitude was established, tetanic stimulation was applied,

and the EPSC amplitude was facilitated (**Fig. 3.10**; n = 27). The facilitation in the presence of 8-bromo-cAMP did not significantly differ from the tetanus-induced facilitation in control conditions ($F_{14,882}$ = 1.18; p = 0.33). Since the effect could not be occluded by increased postsynaptic cAMP, we can conclude that tetanic stimulation does not engage the same pathway as postsynaptic cAMP production. These data are consistent with the hypothesis that tetanus-induced facilitation occurs through presynaptic adenylyl cyclase activation.

G. G-protein activation following tetanus stimulates adenylyl cyclase

Adenylyl cyclase can be directly activated by Ca^{2+}, but it is more commonly activated by specific guanine nucleotide-binding proteins (G-proteins). For these experiments, suramin was used, which acts by uncoupling $G_s\alpha$ subunits from its receptors (Beindl et al., 1996; Freissmuth et al., 1996). Bath application of suramin (200 μM) caused a reduction in EPSC amplitude, and a new baseline EPSC amplitude was established. Following stabilization of EPSC amplitude, tetanic stimulation was performed, and facilitation of the EPSC was completely abolished (**Fig. 3.11**; n=11; $F_{14,658}$ = 4.84; p < 0.0001). These data suggest that tetanic stimulation activates $G_s\alpha$, which stimulates adenylyl cyclase activity, and ultimately leads to facilitation of the EPSC amplitude.

G-protein activation of adenylyl cyclase acts through an interplay of different G-protein subunits. While the $G_s\alpha$ subunit initiates stimulation of adenylyl cyclase activity, inhibitory g-protein subunits ($G_{i/o}\alpha$) act by inhibiting adenylyl cyclase activity. We next determined if blocking $G_{i/o}\alpha$ subunits affected tetanus-induced facilitation. The compound N-ethylmaleimide (NEM; 100 μM) was used, which specifically blocks pertussis-sensitive, $G_{i/o}\alpha$-mediated inhibition (Shapiro et al., 1994). First, we wanted to determine if NEM

application alters the magnitude of forskolin-induced potentiation. The prediction was that if NEM blocks $G_{i/o}\alpha$ -mediated inhibition of adenylyl cyclase, then forskolin-induced potentiation should be enhanced in the presence of NEM. For these experiments, forskolin (10 μM) was first administered by bolus application (duration: 1 minute) to determine the amplitude of forskolin-induced potentiation in control conditions (60.6 ± 6.8%, n = 7; **Fig. 3.12A**). After the forskolin-induced potentiation recovered NEM was added to the bath. NEM caused a potentiation in EPSC amplitude by itself, and a new baseline was established. Next, forskolin was administered again, and a robust potentiation in EPSC amplitude was observed (147 ± 4.3%), which produced a peak amplitude that was significantly greater than the peak amplitude of forskolin-induced potentiation in the absence of NEM ($p < 0.0001$; paired *t* test). These data show that in the presence of NEM, the forskolin-mediated potentiation is enhanced. Next, we wanted to determine the effects of NEM on tetanus-induced potentiation. For these experiments, NEM was bath applied, and once a stable amplitude was established, the tetanic stimulation protocol was applied. Following stimulation, EPSC amplitude potentiated above control conditions, and failed to recover over the course of the experiment (>17 minutes) (**Fig. 3.12B**; n = 5). The magnitude of facilitation of the ESPC in the presence of NEM was significantly greater than that observed in control conditions following tetanic stimulation ($F_{14,574} = 12.104$; $p = 0.001$). Together, these data show that in the presence of NEM, tetanic stimulation produces robust potentiation, which fails to recover.

Since our data indicate that adenylyl cyclase is activated following tetanic stimulation, we next tested the potential role of putative downstream targets of adenylyl cyclase activation, including cAMP and protein kinases. The compound, cAMPS-Rp an

inhibitor of cAMP-induced activation of PKA, was used to determine if the blockade of PKA activation affected the tetanus-induced facilitation of the EPSC. In the presence of cAMPS-Rp (50 μM), tetanic stimulation produced a robust facilitation of the EPSC amplitude that was not significantly different compared to control conditions (**Fig. 3.13A**; n = 6; $F_{15,690}$ = 1.33, p = 0.23). In another series of experiments, we tested if the general protein kinase inhibitor, H7, would alter the tetanus-induced facilitation of the EPSC. In the presence of H7 (10 μM), tetanic stimulation produced a robust facilitation of the EPSC that did not differ from the facilitation in control conditions (**Fig. 3.13B**; n = 13; $F_{15,795}$ = 1.46, p = 0.19). These data indicate that adenylyl cyclase-mediated activation of downstream protein kinases does not contribute to tetanus-induced facilitation.

H. Short-term facilitation is attenuated by K^+ channel blockers

Since the common downstream targets of adenylyl cyclase activation and cAMP production do not appear to contribute to short-term facilitation, we investigated mechanisms that could be involved in altering presynaptic neurotransmitter release. Multiple K^+ channels are located at axon terminals and have been shown to affect glutamate release (Meir et al., 1999; Hille, 2001; Gu and Barry, 2011). The non-selective K^+ channel blocker, tetraethylammonium chloride (TEA) was bath applied. TEA potentiated the EPSC amplitude upon application, so a new baseline EPSC amplitude was attained, and the stimulus intensity was adjusted to ensure that the EPSC amplitude was not at a maximum. After a stable EPSC amplitude was achieved in the presence of TEA (3 mM). Forskolin (10 μM) produced a potentiation of EPSC amplitude (47.34 ± 11.9%; n = 12) that was significantly reduced compared to control forskolin-induced potentiation (**Fig. 3.14A**; $F_{43,602}$ = 7.33, p < 0.0001). Next, we tested if TEA would alter the tetanus-induced

facilitation. In the presence of TEA (3 mM), the tetanus-induced facilitation of the EPSC was completely abolished (**Fig. 3.14B**; n=11; $F_{15,765} = 4.6$; $p < 0.0001$). While facilitation was reduced, there was a small magnitude of suppression in EPSC amplitude observed following stimulation (21.4 ± 5.8%; $p = 0.003$; paired *t* test).

Since the experiments in the presence of TEA confirm the contribution of K^+ channels to short-term facilitation, we narrowed down the possibilities by specifically blocking voltage-gated K^+ channels with 4-AP (100 μM). 4-AP wash in caused a large potentiation in EPSC amplitude, and the stimulus intensity was adjusted to ensure that the EPSC amplitude was not at a maximum. A new baseline EPSC amplitude was established, and the stimulation protocol was applied. Following stimulation, all cells failed to show facilitation (**Fig. 3.14C**; n = 7; $F_{15,705} = 8.1$, $p < 0.0001$), and EPSC amplitude was significantly reduced (37 ± 12.1%) compared to control conditions. These findings show that blockade of voltage-gated K^+ channels prevents potentiation following tetanic stimulation; however, it also produces a lasting suppression in EPSC amplitude. From these results, we can conclude that voltage-gated K^+ channels contribute to tetanus-induced potentiation.

I. Short-term facilitation is mediated by I_h

Thus far, we have shown that K^+ channels, acting downstream of adenylyl cyclase, are involved in tetanus-induced facilitation of EPSCs. Since cAMP production is also downstream of adenylyl cyclase, and cAMP can directly activate ion channels, we wanted to determine the contribution of cAMP-activated ion channels to tetanus-induced facilitation. We first looked at the effects of cesium chloride (Cs Cl, 3mM), which is an inhibitor of the hyperpolarization-activated cyclic nucleotide modulated (HCN) current (I_h). Bath

application of CsCl potentiated EPSC amplitude, and the stimulus intensity was adjusted to ensure that the EPSC amplitude was not at a maximum. After a new baseline EPSC amplitude was established, tetanic stimulation was applied. In the presence of CsCl, tetanic stimulation produced facilitation of EPSC amplitude (37.8 ± 14.0%), which did not recover for the remainder of the experiments (> 8 minutes). Although tetanic stimulation produced some facilitation, the EPSC amplitude was significantly reduced, compared to control conditions (**Fig. 3.15A**; n = 9; $F_{14,630} = 3.71$; $p = 0.001$). These results suggest that HCN channels could potentially contribute to tetanus-induced facilitation. However, more specific pharmacology experiments were done to determine the contribution of I_h.

To specifically determine if I_h contributes to short-term facilitation, the effects of the selective I_h blocker, ZD 7288 (50μM), were tested. ZD 7288 was bath applied, and tetanic stimulation was given. In the presence of ZD 7288, all cells failed to show facilitation in EPSC amplitude (**Fig. 3.15B**; n = 12). When compared to the control stimulation condition, EPSC amplitude following tetanic stimulation was significantly reduced ($F_{14,672} = 5.133$; $p < 0.0001$). While the cells did not show facilitation, suppression of EPSC amplitude was evident approximately 1-2 minutes after stimulation (45.7 ± 5.8%; $p < 0.0001$; paired *t* test). These data indicate that I_h plays a role in the tetanus-induced potentiation.

3.4 Discussion

In this study, we described the mechanism of short-term plasticity in thalamic relay neurons following tetanic stimulation of corticothalamic afferents. Using high-frequency tetanic stimulation of corticothalamic afferents, short-term facilitation could reliably be induced in the majority of thalamic relay neurons (81%; n = 34/42).

Following tetanic stimulation, relay neurons displayed a 108.6 ± 7.7% increase in magnitude of EPSC amplitude. The large magnitude increase of corticothalamic output, which persists for approximately 20 minutes before recovering, may act to transiently and effectively enhance information flow through the thalamocortical circuit, and thereby regulate the transfer of salient information to the cortex. Short-term plasticity could be a potential gain control mechanism instilled by the cortex to enhance future sensory inputs, and regulate thalamic output. Facilitation in corticothalamic synaptic efficacy could not only enhance sensory information flow to the cortex, but it could also facilitate cortico-cortical communication via the thalamus.

Our experiments suggest that short-term facilitation is presynaptic. We showed that both NMDAR- and AMPAR-mediated currents display potentiation. Since both of these glutamatergic receptors are primarily located postsynaptically, these suggested a presynaptic site of action. Next, we showed that paired-pulse ratio decreases following tetanic stimulation. Studies show that a change in paired-pulse ratio is indicative of a presynaptic mechanism of action (Schulz et al., 1994), which is further evidence that our effect is due to increased presynaptic neurotransmission.

Our next series of experiments specify that short-term facilitation is Ca^{2+}-dependent, since facilitation could be blocked in the absence of Ca^{2+}. These data were especially indicative of a Ca^{2+}-dependent effect, since facilitation could be reestablished in the same cell, following wash in of physiological saline. Additionally, the magnitude of facilitation was similar to that of control conditions.

Through a series of adenylyl cyclase activation and occlusion experiments using forskolin, we were able to show that short-term facilitation is engaging the adenylyl cyclase

signaling pathway. Blockade of adenylyl cyclase activity with SQ-22536 also supported these results.

Since our data suggest that short-term facilitation is presynaptic and Ca^{2+}-dependent, we hypothesize that Ca^{2+} activation of adenylyl cyclase is initiating the signaling cascade. High-frequency stimulation could cause influx of Ca^{2+} or induce release of Ca^{2+} from intracellular stores. Previous findings have shown that Ca^{2+} binding to calmodulin can directly activate adenylyl cyclase, via specific Ca^{2+}-stimulated isoforms of adenylyl cyclase (MacNeil et al., 1985). Therefore, activation of these isoforms by Ca^{2+} could initiate the intracellular adenylyl cyclase signaling pathway. We also showed that G-protein actions could also contribute to adenylyl cyclase activation, by enhancing $G_s\alpha$- or inhibiting $G_{i/o}\alpha$ -mediated effects. We show that blocking $G_s\alpha$ with suramin inhibited tetanus-induced facilitation. Interestingly, blocking $G_{i/o}\alpha$ activity with NEM produced facilitation, which did not display recovery. One possibility is that $G_s\alpha$ and $G_{i/o}\alpha$ are both contributing to the trend of facilitation that is observed following stimulation. $G_s\alpha$ activation of adenylyl cyclase could be responsible for the initial increase in EPSC amplitude, and as a compensatory mechanism, $G_{i/o}\alpha$ may become activated over time, to suppress the potentiation and facilitate recovery to baseline levels. Therefore, $G_{i/o}\alpha$ activation may contribute to the time duration of tetanus-induced plasticity.

Next, we investigated the downstream effectors of adenylyl cyclase activation and subsequent cAMP production. We showed that protein kinases are not activated downstream of cAMP to impact short-term facilitation. We consistently show these results, in which neither protein kinase inhibitor tested (cAMPS-Rp or H7) blocked short-term facilitation. These findings are consistent with theories that protein kinase activation and

subsequent phosphorylation of downstream transcription factors (i.e. CREB, mTOR, etc.) are primarily evident in long-term plasticity changes (Bear and Malenka, 1994; Alberini, 2009).

To determine the downstream effect of adenylyl cyclase activation, we examined factors that could influence enhanced neurotransmitter release, aside from activation of protein kinases. Ion channel dynamics have been shown to modulate presynaptic neurotransmitter release (Meir et al. 1999), so we investigated the contribution of K^+ channels on tetanus-induced facilitation. TEA and 4-AP, two K^+ channel blockers, significantly reduced tetanus-induced facilitation, demonstrating the involvement of K^+ channels in this process. We hypothesize that a change in K^+ channel conductance could lead to enhanced glutamatergic release. Interestingly, while TEA and 4-AP both blocked facilitation, they showed different effects. Cells that received tetanic stimulation in the presence of TEA showed a small magnitude of suppression; however, in the presence of 4-AP, cells showed a dramatic suppression in EPSC amplitude following stimulation. Both agents block several types of K^+ channels, but 4-AP mainly blocks voltage-gated K^+ channels, including non-inactivating delayed rectifier channels and A-type K^+ channels (Meves and Pichon, 1977; Thompson, 1982). TEA, on the other hand, blocks delayed rectifiers and A-type channels, as well as inward rectifiers, and Ca^{2+}-activated K^+ channels (Standen and Stanfield, 1980; Farley and Rudy, 1988; Foehring and Surmeier, 1993). One possibility is that Ca^{2+} produced from tetanic stimulation activates Ca^{2+}-activated K^+ channels to suppress neurotransmitter release, which is observed specifically in the presence of 4-AP. While inward rectifiers may also contribute to the suppression, there has

not been much evidence of inward rectifying K^+ channels in mammalian axon terminals, while Ca^{2+}-activated K^+ channels are more prevalent.

Suppression of EPSC amplitude was also evident to similar magnitudes following tetanic stimulation in the Ca^{2+}-free and ZD 7288 experiments. Since suppression was observed in the absence of Ca^{2+}, it could potentially be inferred that Ca^{2+}-activated K^+ channels do not contribute to the decrease in EPSC amplitude, if the suppression that is consistently observed is acting through similar mechanisms. Tetanic stimulation is causing suppression of EPSC amplitude in some of these experimental paradigms, but suppression is likely masked by the large magnitude of potentiation that is induced in control conditions. The suppression of EPSC amplitude may determine the overall magnitude of potentiation.

Both A-type and delayed rectifier K^+ channels have been observed at axon terminals of several species, and contribute to presynaptic neurotransmitter release (Sheng et al., 1993; Moreno et al., 1995; Veh et al., 1995; Weiser et al., 1995; Ponce et al., 1996). Therefore, these K^+ channels could contribute to tetanus-induced facilitation at corticothalamic synapses. We hypothesize that tetanic stimulation leads to the closure of specific K^+ channels, which leads to depolarization of the axon terminal. Terminal depolarization can then cause enhanced neurotransmitter release. This effect can persist as long as adenylyl cyclase is activated, and recovery could occur once adenylyl cyclase activity reaches basal levels and K^+ channels are reopened. Ca^{2+} and G-protein-mediated activation of adenylyl cyclase could be a mechanism by which adenylyl cyclase can sustain facilitation for ~20 minutes. This could also be a mechanism by which neurotransmitter output can be altered based on quick regulation of Ca^{2+} levels or $G_s\alpha$ activation.

Another mechanism through which K^+ channels could perpetuate an effect that lasts for approximately 20 minutes, involves feed forward depolarization from other channels. The closure of K^+ channels has been shown to directly increase Ca^{2+} channel conductance at axon terminals, altering neurotransmission (Meir et al., 1999; Hille, 2001). This causes a feed forward depolarization, which may secondarily contribute to the longer lasting duration of synaptic facilitation, without requiring the persistent activation of adenylyl cyclase.

We also showed how tetanus-induced facilitation is due to the influence of I_h, since CsCl and ZD 7288 both block short-term facilitation. Together, these data suggest that I_h activates an inward current, which leads to the depolarization of axon terminals, and subsequently enhancing neurotransmitter release. I_h has been shown to act at axon terminals to modulate presynaptic release (Bender and Baram, 2008). HCN channels are mainly activated by hyperpolarization, but are modulated by cAMP; cAMP can shift the voltage-dependence of activation of HCN channels to more depolarized potentials, and increase the rate of channel opening (Craven and Zagotta, 2006; Kase and Imoto, 2012). We show that adenylyl cyclase activation occurs following tetanic stimulation, leading to cAMP production; cAMP may directly bind to HCN channels to shift voltage-dependency, and cause depolarization of the terminal by HCN channel opening.

Several studies have shown how I_h is often activated with other conductances to impact information processing in neurons (George et al., 2009; Huang et al., 2011; Kase and Imoto, 2012); K^+ and HCN channels could work together to contribute to altered terminal dynamics at corticothalamic synapses, as our results indicate.

Together, out data allows us to form a comprehensive hypothesis about the mechanism of short-term facilitation (**Fig. 3.16**). Tetanic stimulation causes increased intracellular Ca^{2+} levels at presynaptic corticothalamic terminals. Ca^{2+} and G-proteins directly activate adenylyl cyclase, which produces elevated cAMP levels. cAMP can indirectly lead to the closure of K^+ channels, and directly modulate HCN channels. Both of these effects act to depolarize the axon terminal, leading to increased glutamatergic release.

Short-term facilitation in the thalamocortical circuit has major implications with respect to sensory processing, since transiently increasing corticothalamic excitatory output can affect how relay neurons integrate and transfer incoming sensory information. Increased corticothalamic drive can effectively modulate gain control in relay neurons, by influencing relay neuron excitability and how it integrates incoming sensory information. Corticothalamic neurons could act to regulate efficient control of the relay neuron by rapidly adjusting synaptic efficacy on a moment-to-moment basis, depending on environmental demands. Understanding the cellular mechanism of short-term synaptic plasticity can form a basis to understand further behavioral implications of corticothalamic feedback in sensory processing and perception.

APPENDIX

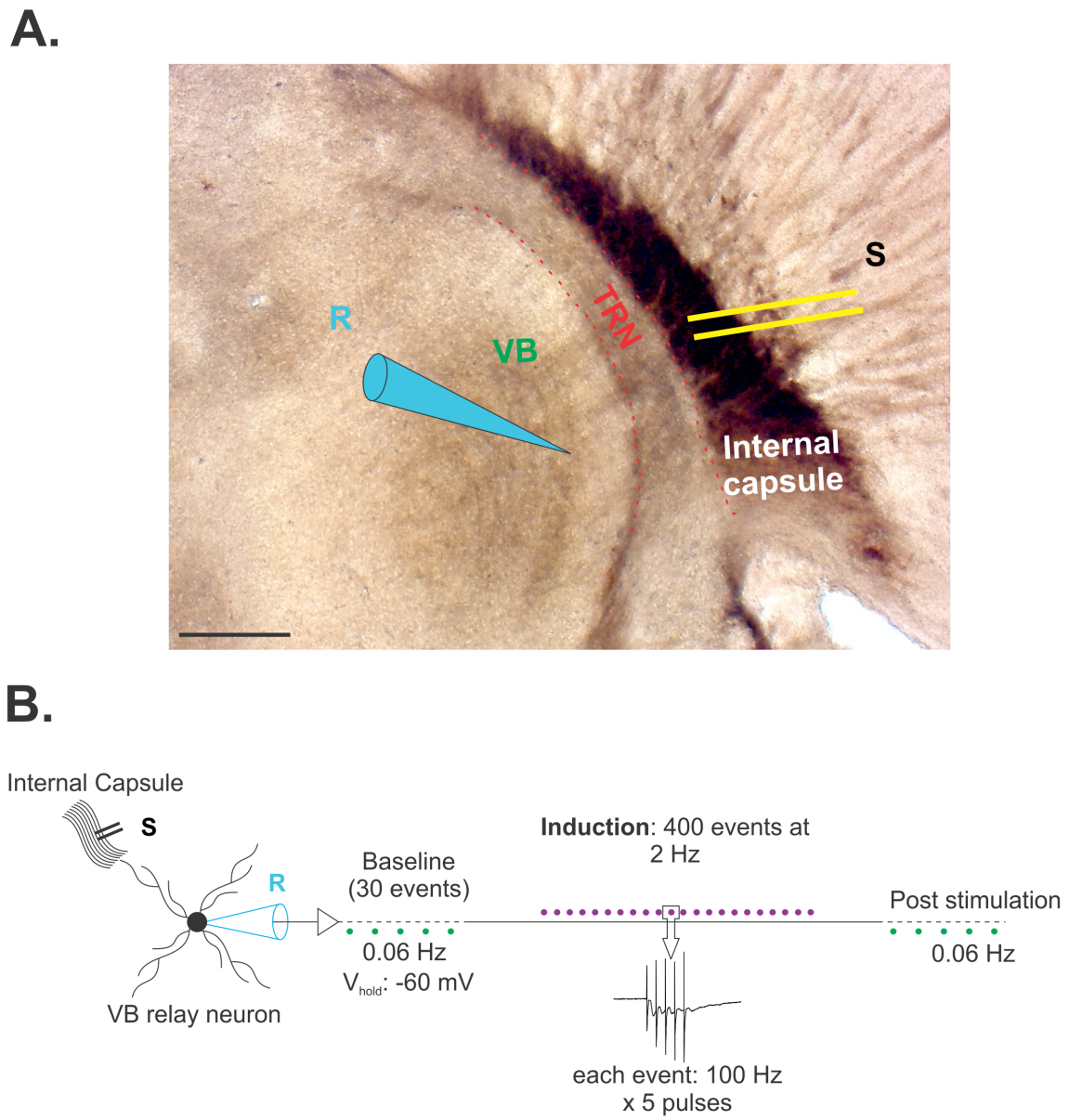

Figure 3.1. Brain slice and induction paradigm used to produce short-term plasticity at corticothalamic synapses

A. Image of the horizontal thalamic slice used, indicating placement of recording (R) and stimulating (S) electrode. Scale bar = 500 µm. **B.** Tetanic stimulation paradigm: EPSCs were evoked at 15-second intervals (0.06 Hz) and at least 30 consecutive, stable responses were recorded (green dots) prior to the induction protocol. Induction involved tetanic stimulation (5 pulses at 100 Hz), repeated 400 times at 2 Hz (purple dots). Following induction, EPSCs were evoked at 15-second intervals.

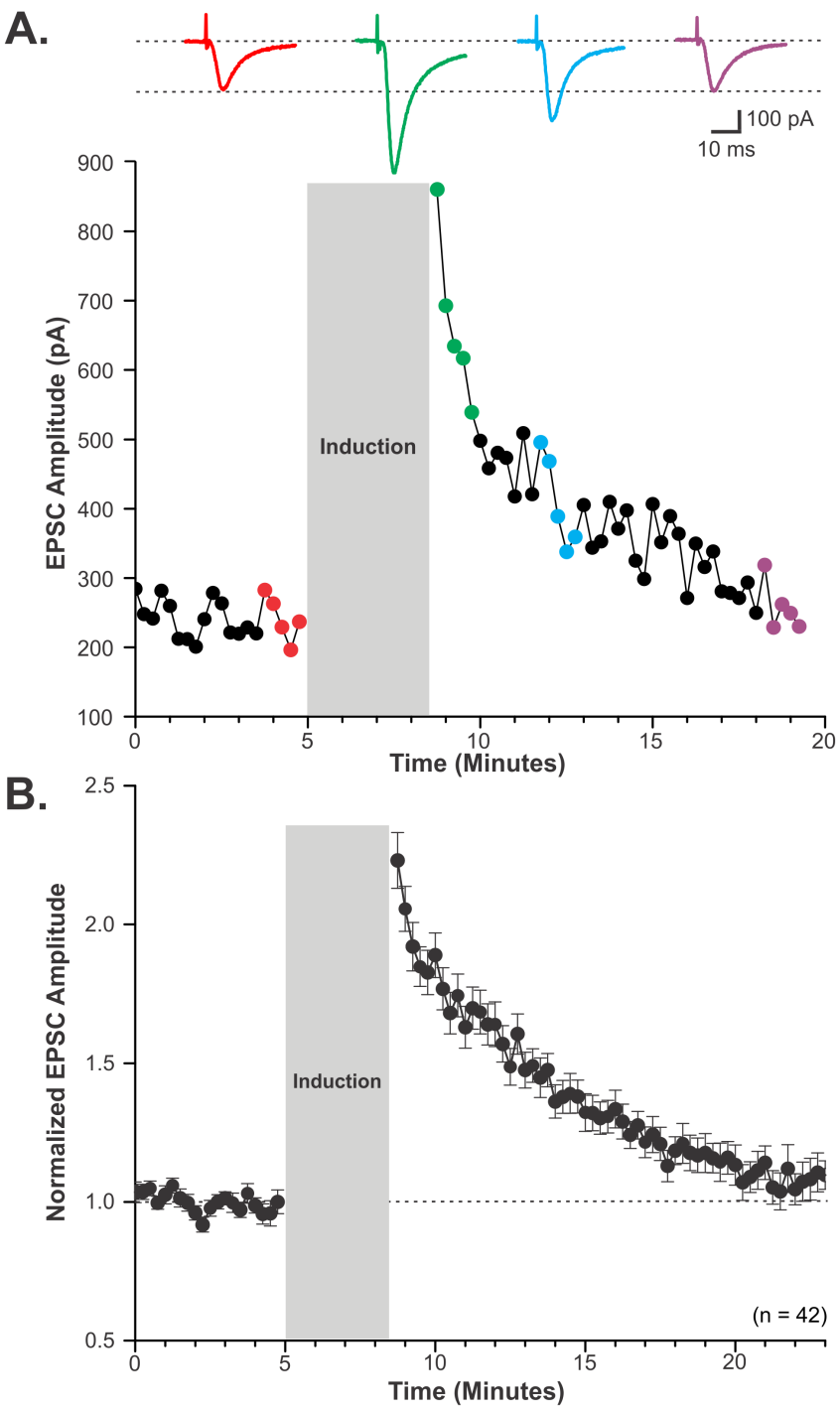

Figure 3.2. Tetanic stimulation of corticothalamic synapses produces short-term facilitation of EPSC amplitude

A. Representative responses, displaying short-term facilitation. *Top*, Representative EPSCs (average of 5 consecutive responses) before tetanic stimulation (red) and at three time points following the induction protocol. *Bottom*, Graph of a representative recording, displaying short-term facilitation of EPSC amplitude following induction. **B.** Graph of population data, indicating that 81% of cells (34 of 42) displayed short-term facilitation.

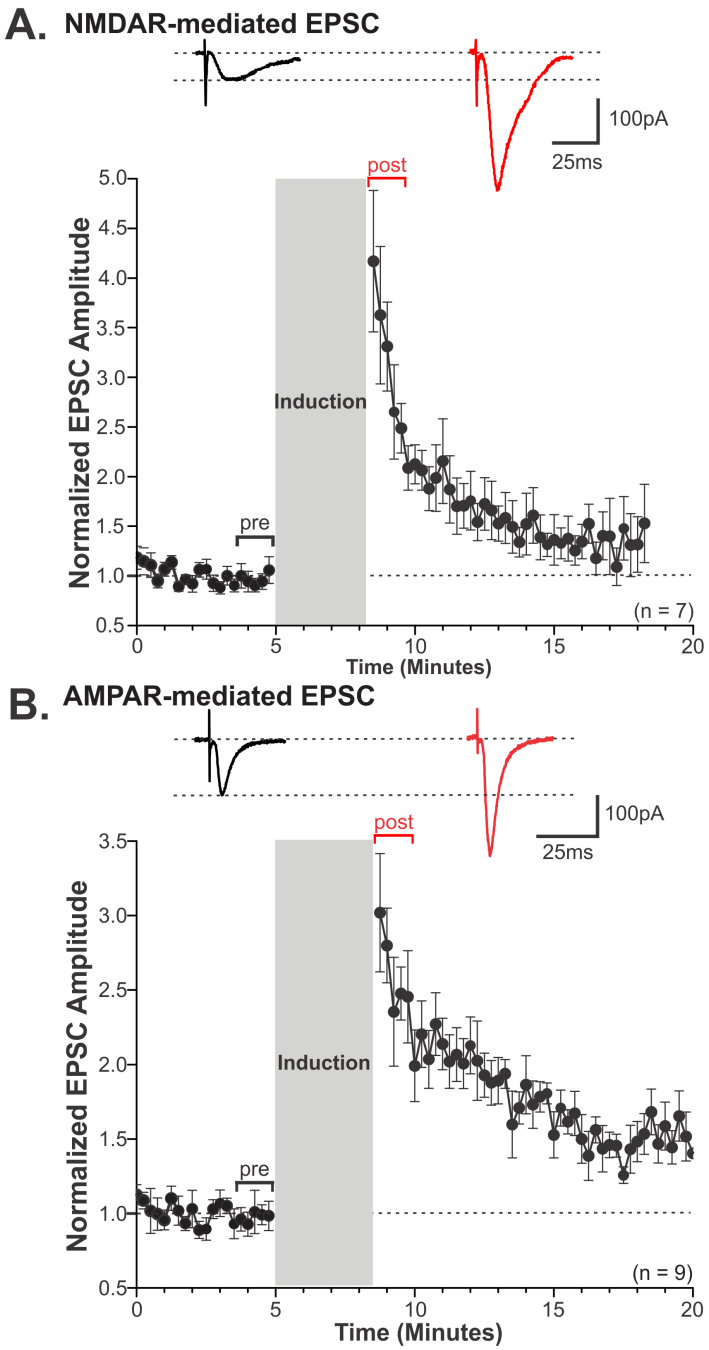

Figure 3.3. Tetanic stimulation facilitates AMPAR- and NMDAR- mediated currents
A. NMDAR-mediated EPSCs display short-term facilitation following tetanic stimulation. *Top*, Representative NMDAR-mediated EPSCs (average of 5 consecutive responses) recorded at V_{hold}: -25 mV, and isolated with DNQX (20 μM). *Bottom*, Graph of population data of all NMDAR-mediated EPSCs recorded (n = 7), showing short-term facilitation following tetanic stimulation. **B.** AMPAR-mediated EPSCs display short-term facilitation following tetanic stimulation. *Top*, Representative AMPAR-mediated EPSCs (average of 5 consecutive responses) recorded at V_{hold}: -60 mV, and isolated with CPP (10 μM). *Bottom*, Graph of population data of all AMPAR-mediated EPSCs recorded (n = 9), showing short-term facilitation following tetanic stimulation.

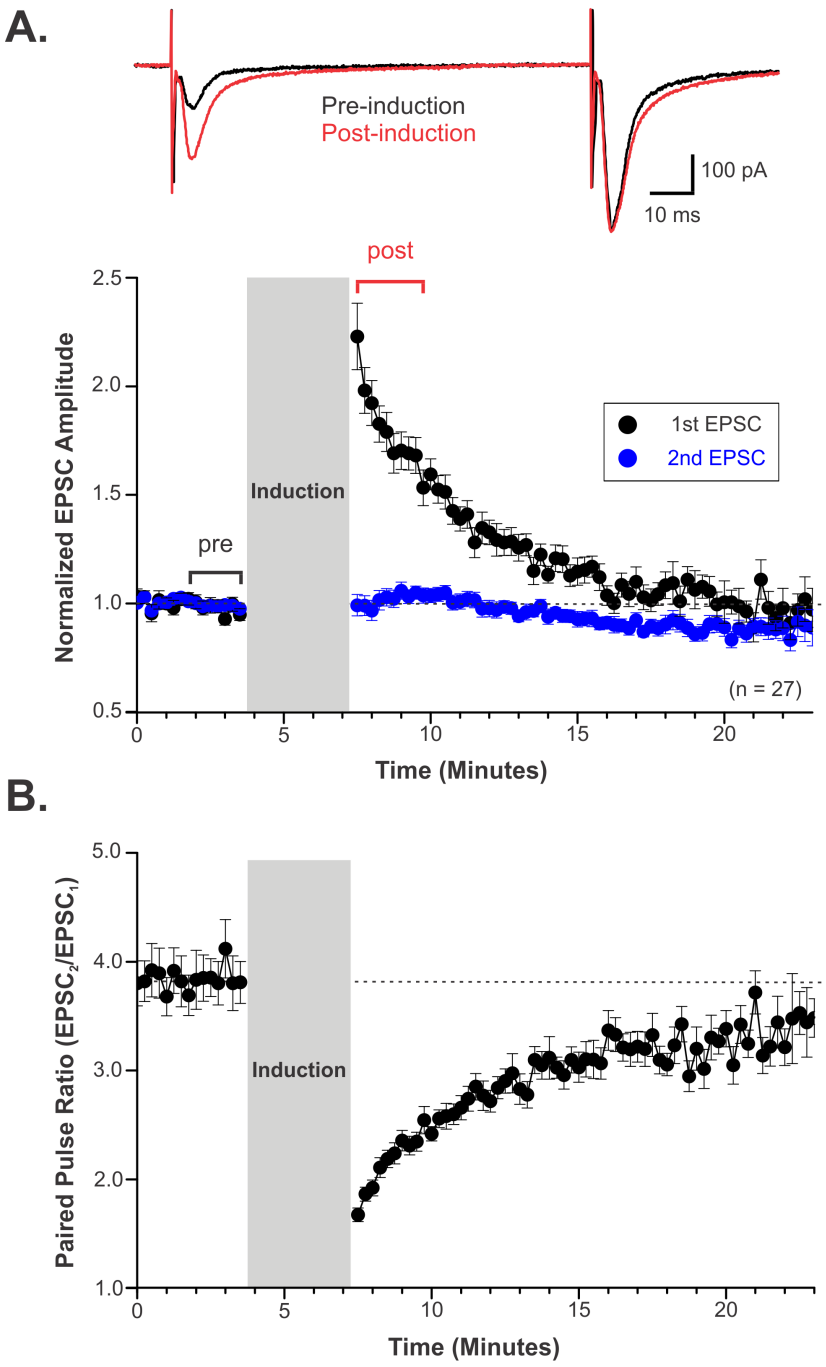

Figure 3.4. Paired-pulse ratio is decreased following tetanic stimulation
A. Tetanic stimulation causes facilitation of $EPSC_1$. *Top*, Representative EPSCs showing changes in paired-pulse facilitation (Black: pre-induction; red: post induction, interstimulus interval: 100 ms). *Bottom*, Graph of population data showing the facilitation in $EPSC_1$ following the induction protocol, and no alteration in $EPSC_2$. **B.** Paired-pulse ratio ($EPSC_2/EPSC_1$) decreases following induction (n = 27; p = 0.018; paired *t* test).

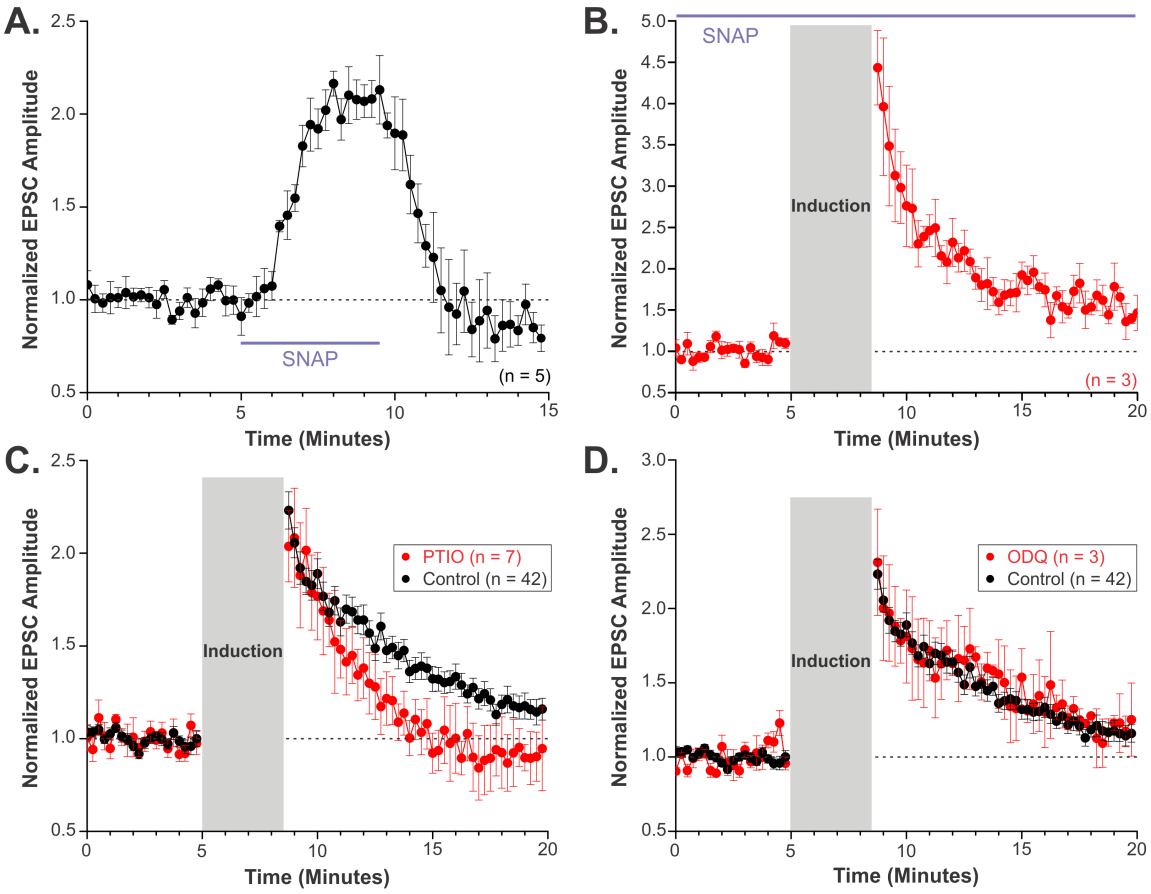

Figure 3.5. Short-term facilitation is not mediated by NO signaling
A. Potentiation in EPSC amplitude is observed following application of the NO donor, SNAP (500 μM, 3.75 minutes). SNAP application produces an increase in EPSC amplitude (n = 5). **B.** SNAP occlusion experiments were performed, where tetanic stimulation was applied in the presence of SNAP, which produced robust facilitation in EPSC amplitude. **C.** Tetanic stimulation in the presence of NO inhibitor, PTIO (20 μM), causes facilitation in EPSC amplitude, which does not differ from control conditions (n = 7; p = 0.30). **D.** Tetanic stimulation in the presence of the guanylyl cyclase inhibitor, ODQ (50-100 μM), causes facilitation in EPSC amplitude, which does not differ from control conditions (n = 3; p = 0.10).

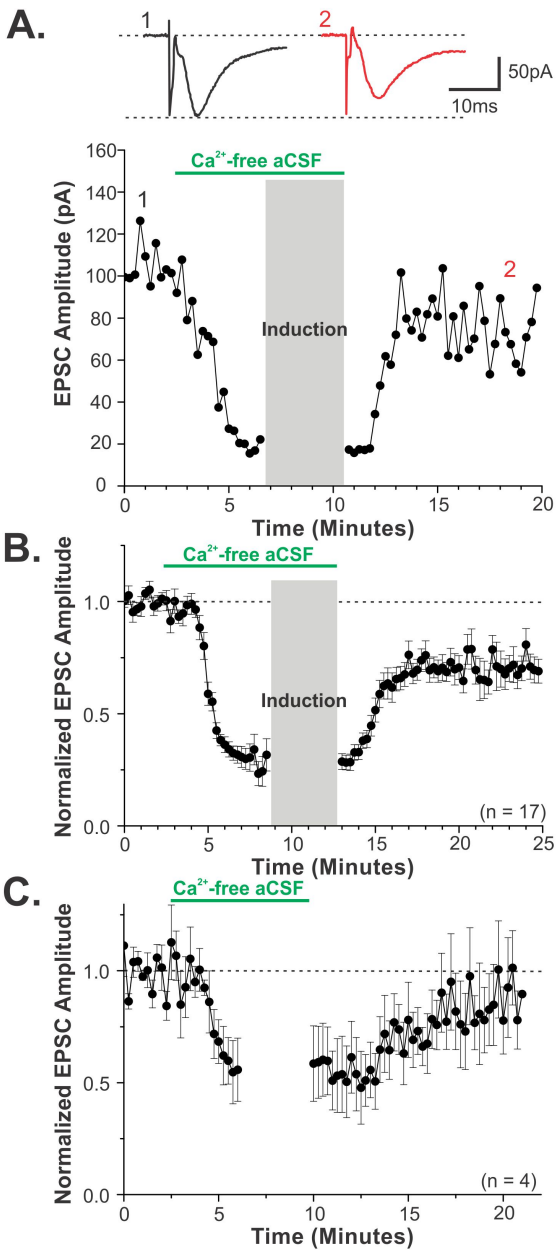

Figure 3.6. Short-term facilitation is Ca^{2+}-dependent
A. Representative responses, showing the absence of facilitation in Ca^{2+}-free artificial cerebrospinal fluid (aCSF) (0mM Ca^{2+}, 4 mM Mg^{2+}). *Top*, Representative EPSCs (average of 10 consecutive responses) of EPSCs showing the absence of facilitation following stimulation. *Bottom,* Graph of a representative recording, showing the absence of facilitation following tetanic stimulation with Ca^{2+}-free aCSF. **B.** Graph of population data, indicating that tetanic stimulation in the presence of Ca^{2+}-free aCSF did not produce facilitation (n = 17), and suppression was evident. **C.** Ca^{2+}-free control experiments were run, where Ca^{2+}-free aCSF was administered for the same duration as in *B.*, but without tetanic stimulation. Cells that received Ca^{2+}-free wash in alone displayed suppression of EPSC amplitude (n = 4).

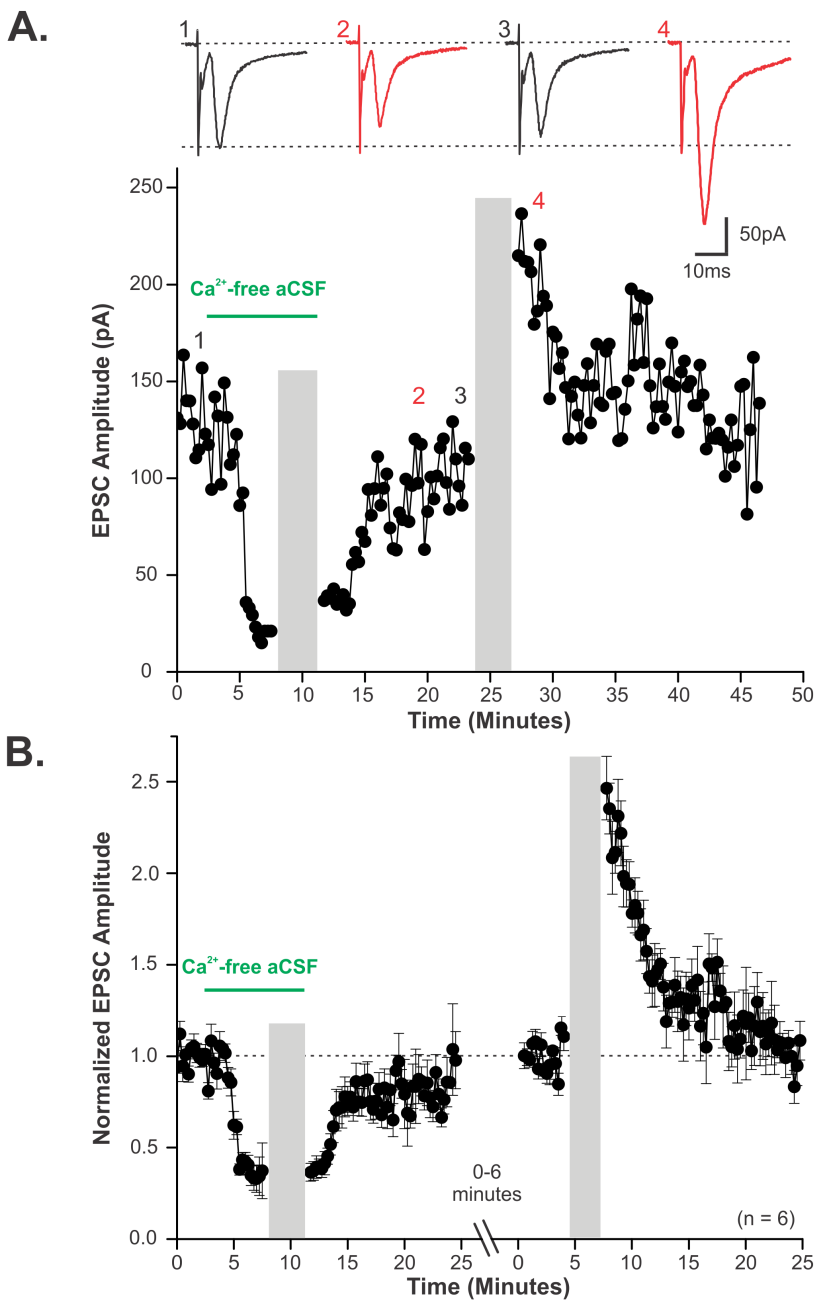

Figure 3.7. Short-term facilitation can be abolished in the absence of extracellular Ca^{2+} and reestablished in the presence of Ca^{2+}

A. Representative responses, showing the absence of tetanus-induced facilitation in Ca^{2+}-free aCSF, followed by short-term facilitation after tetanic stimulation. *Top*, representative EPSCs (average of 10 consecutive responses). *Bottom*, graph of a representative recording, showing the absence of facilitation after tetanic stimulation in Ca^{2+}-free aCSF, followed by reestablished short-term facilitation after tetanic stimulation in control aCSF. **B.** Graph of population data, showing the absence of facilitation in Ca^{2+}-free aCSF, followed by robust facilitation after stimulation in control aCSF ($p = 0.94$; unpaired *t* test). Gray bars indicate time of induction protocol.

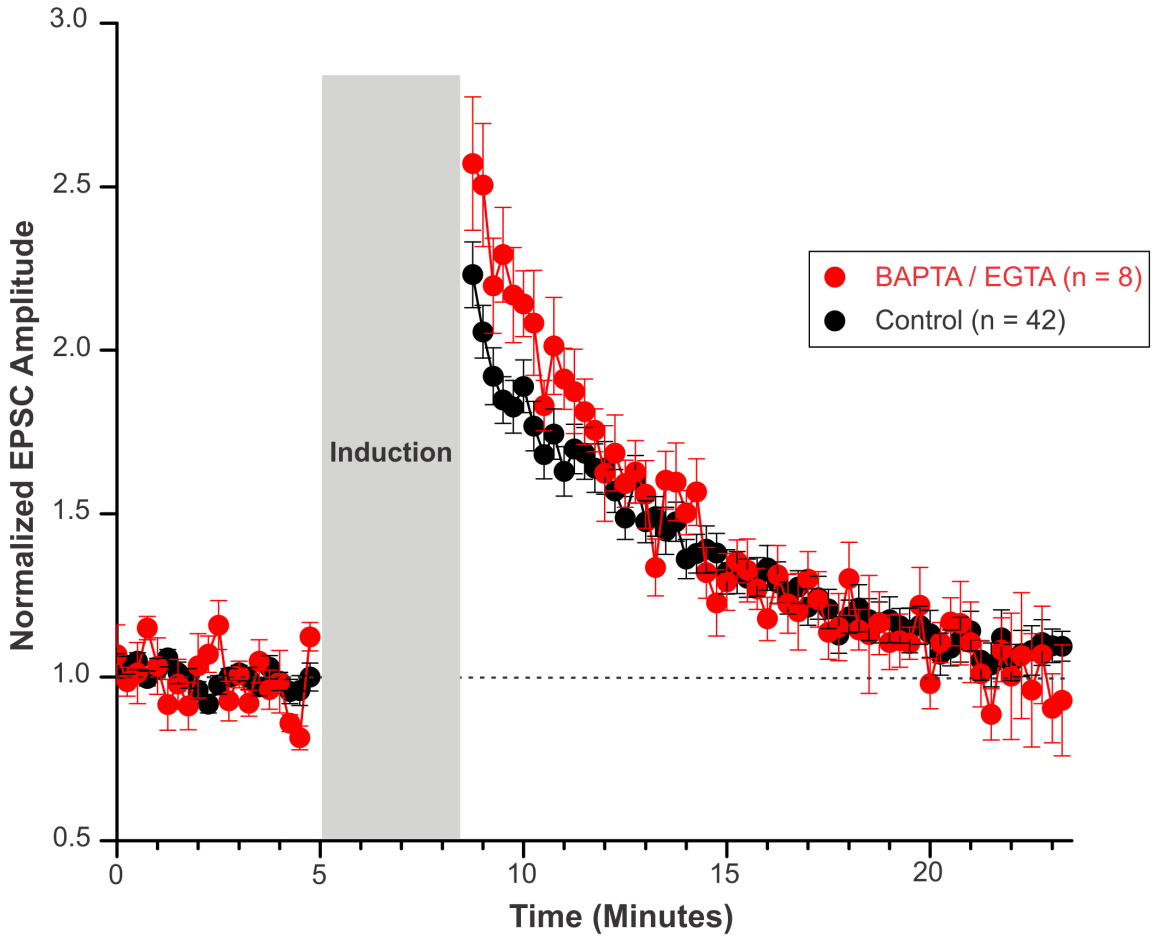

Figure 3.8. Intracellular Ca^{2+} chelators do not affect short-term facilitation
Intracellular Ca^{2+} chelators, BAPTA (10 mM) and EGTA (2 mM), were included in the recording pipette, and the population data show a robust facilitation in EPSC amplitude, which did not significantly differ from control conditions (n = 8; p = 0.10).

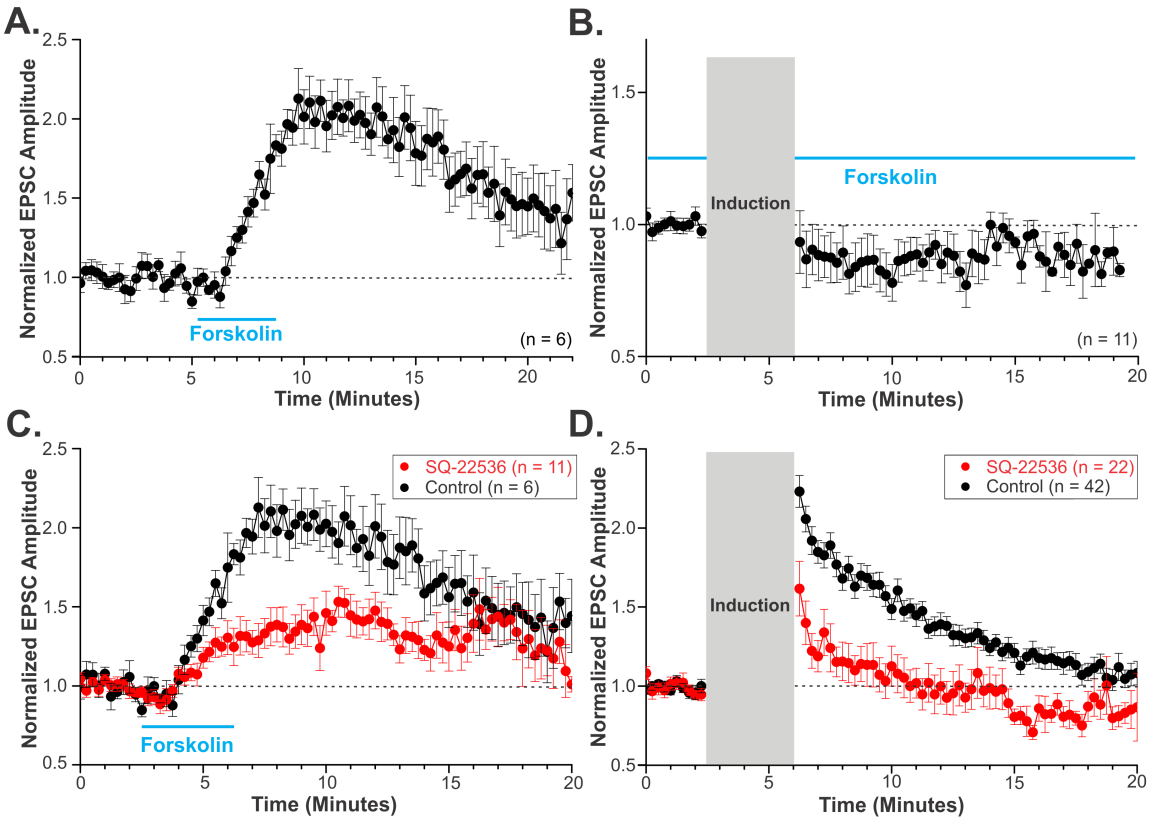

Figure 3.9. Short-term facilitation is mediated by adenylyl cyclase activation
A. Forskolin application (10 μM; 3.75 minutes) induces potentiation of EPSC amplitude. **B.** Forskolin occlusion experiments were performed, where tetanic stimulation was applied in the presence of forskolin. All cells failed to show facilitation. **C.** Forskolin application in the presence of the adenylyl cyclase inhibitor, SQ-22536 (50 μM), produced an attenuated potentiation in EPSC amplitude compared to control conditions ($p < 0.0001$). **D.** In the presence of SQ-22536, tetanus-induced facilitation was significantly reduced, compared to control conditions (n = 22; $p = 0.018$).

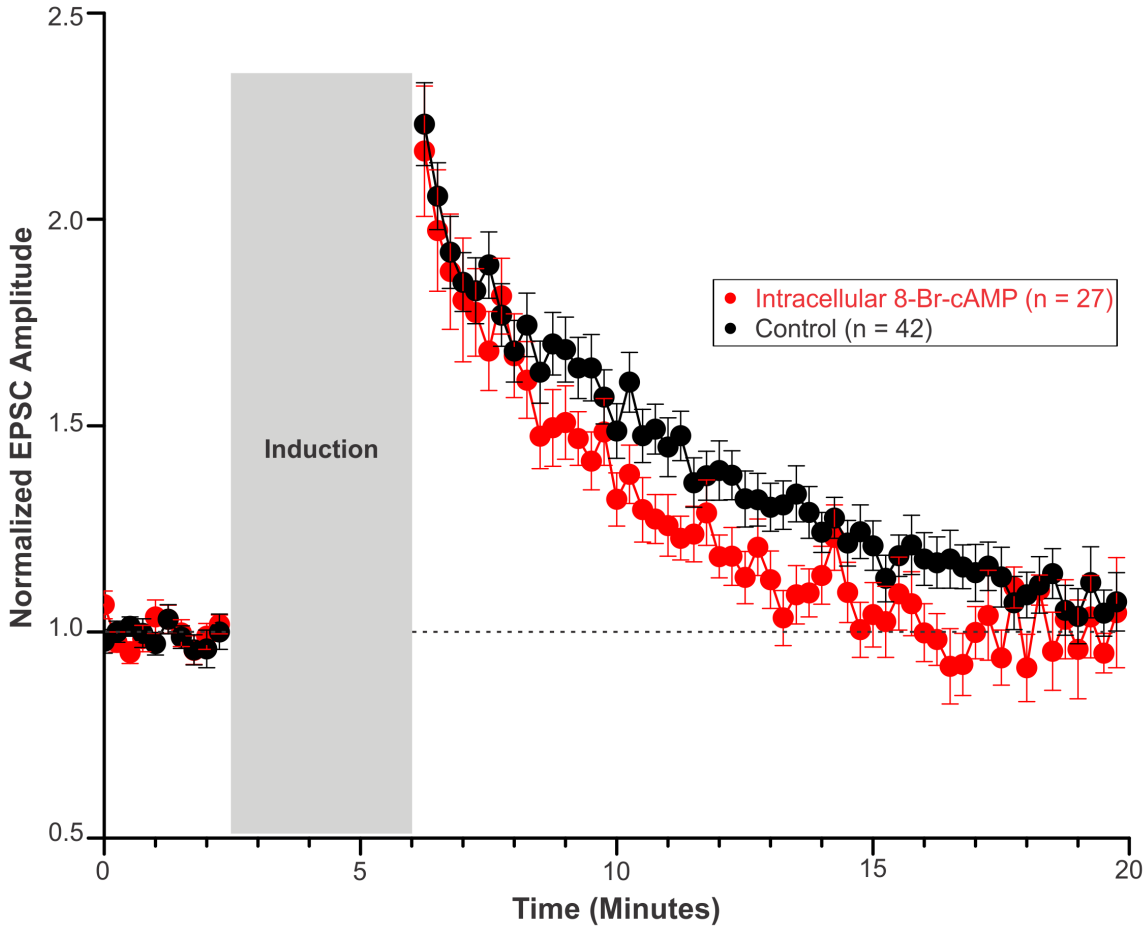

Figure 3.10. Intracellular 8-bromo-cAMP does not affect short-term facilitation
Intracellular cAMP analogue, 8-bromo-cAMP (10 mM) was included into the recording pipette, and the population data show a robust facilitation in EPSC amplitude, which did not significantly differ from control conditions (n = 27; p = 0.29).

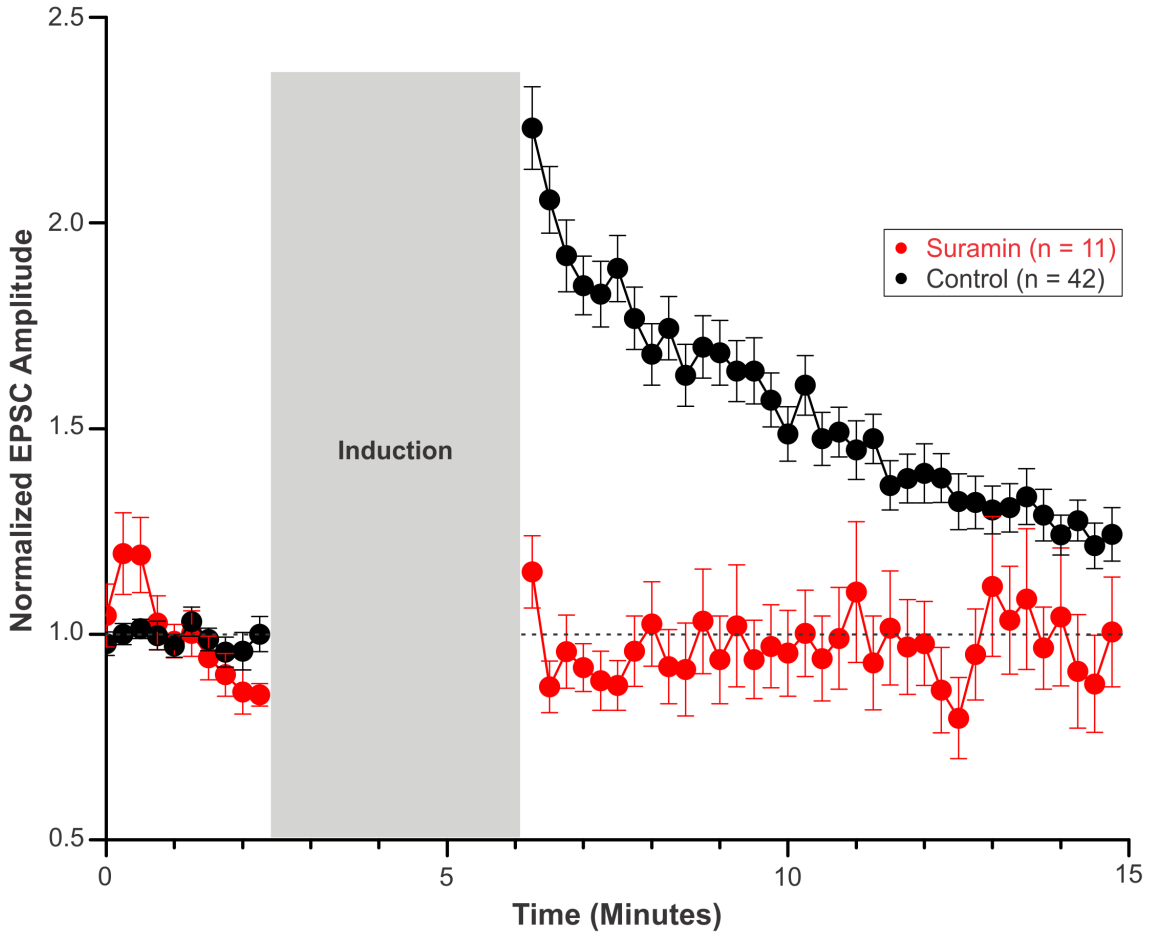

Figure 3.11. Short-term facilitation is mediated by G-protein activation
Tetanic stimulation in the presence of $G_s\alpha$ inhibitor, suramin (200 μM) blocks facilitation completely (n = 11; $p < 0.0001$).

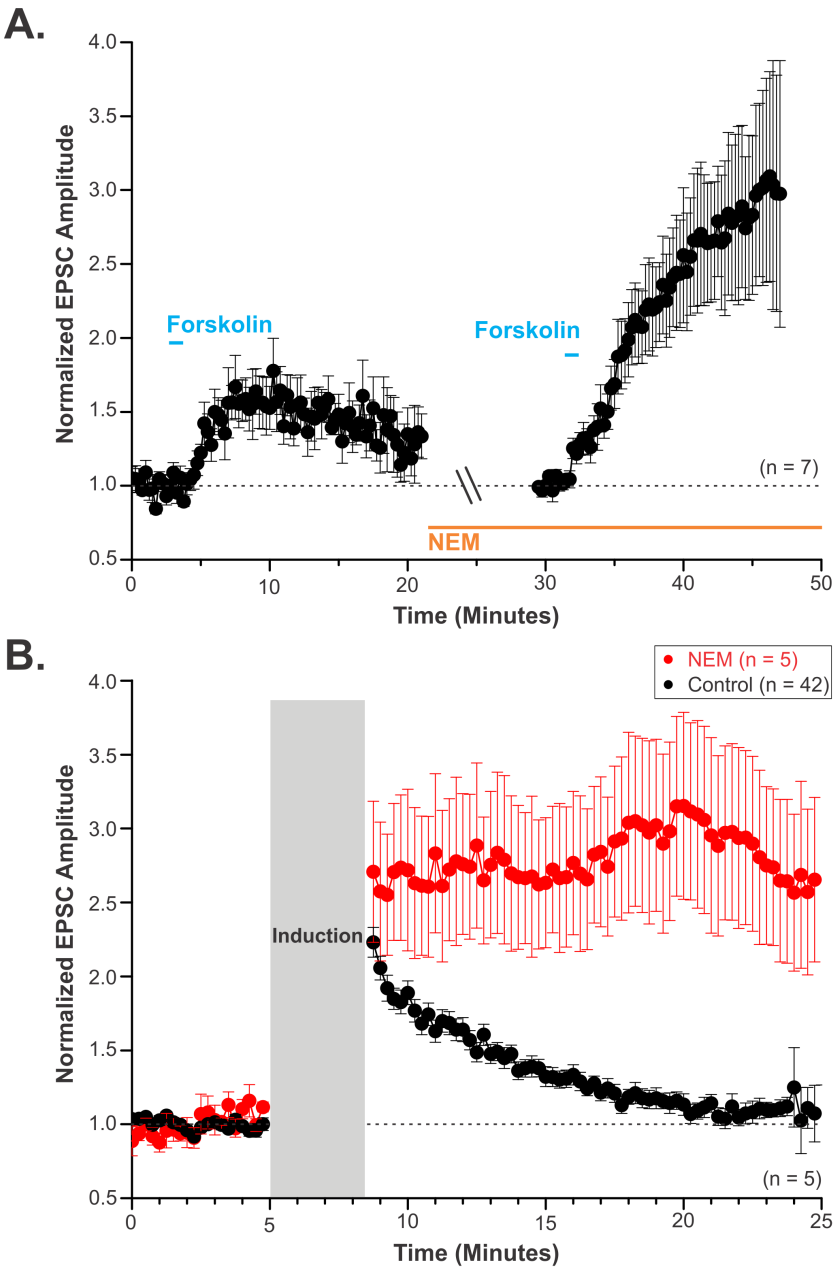

Figure 3.12. NEM-mediated $G_{i/o}\alpha$ inhibition prolongs facilitation
A. The $G_{i/o}\alpha$ inhibitor, N-ethylmaleimide (NEM, 100 μM) enhances forskolin-mediated potentiation. Forskolin (10 μM; 1 minute) was applied to determine the magnitude of forskolin-induced potentiation. Next, NEM was added to the bath, which caused a potentiation in EPSC amplitude. Break in the dashed line signifies where the baseline was renormalized to 1. Forskolin was reapplied in the presence of NEM, and the forskolin-mediated potentiation was significantly enhanced ($p < 0.0001$, paired t test). **B.** Tetanic stimulation in the presence of NEM produced robust facilitation, which did not recover (> 17 minutes), and was significantly greater than facilitation in control conditions ($p = 0.001$).

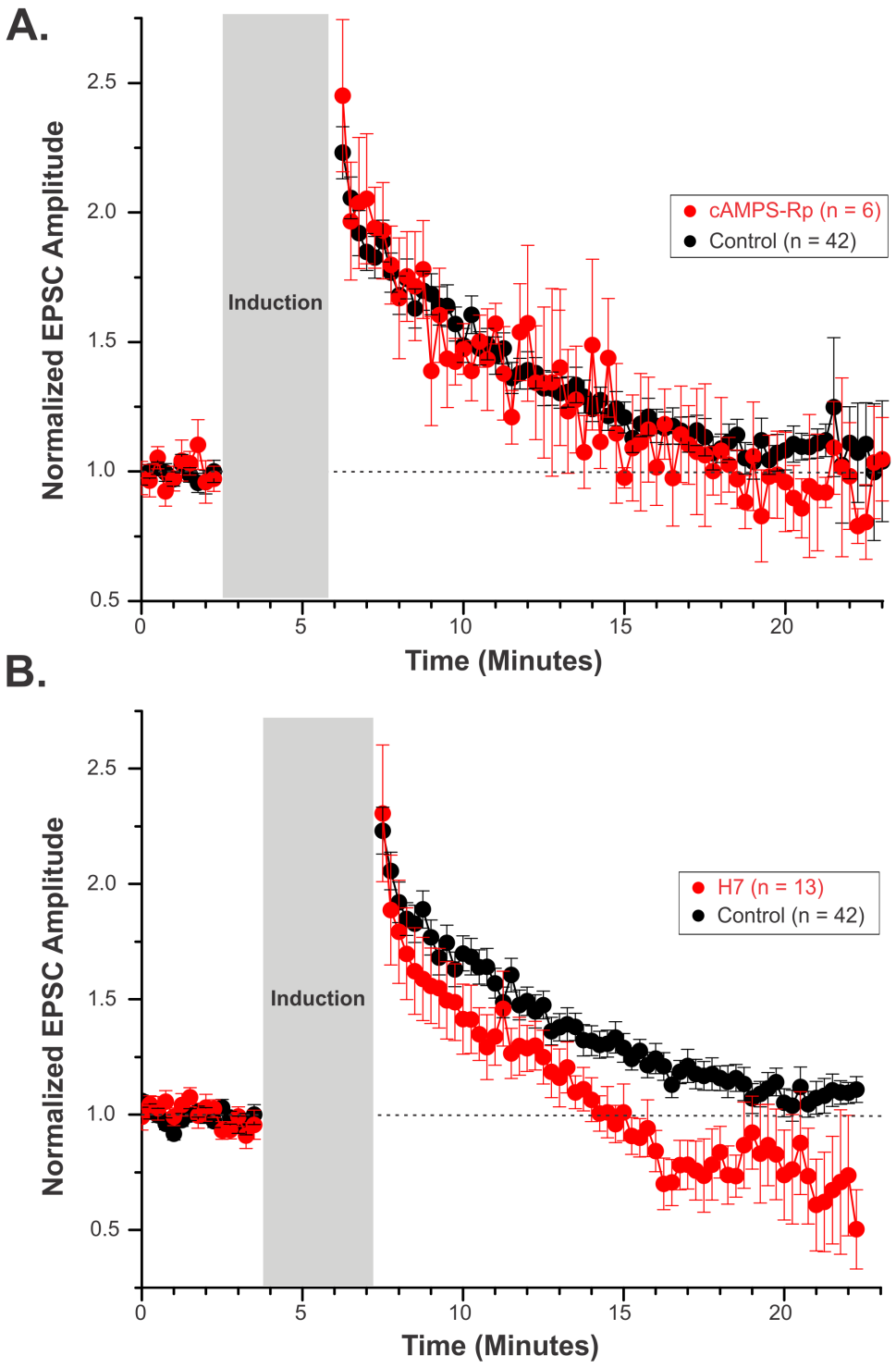

Figure 3.13. Protein kinase inhibitors do not affect short-term facilitation
A. Tetanic stimulation in the presence of the PKA inhibitor, cAMPS-Rp (50 μM) caused facilitation of EPSC amplitude, which did not significantly differ from control conditions (n = 6; $p = 0.13$). **B.** Next, tetanic stimulation in the presence of the general protein kinase inhibitor, H7 (10 μM) produced facilitation of EPSC amplitude, which did not significantly differ from control conditions (n = 13; $p = 0.11$).

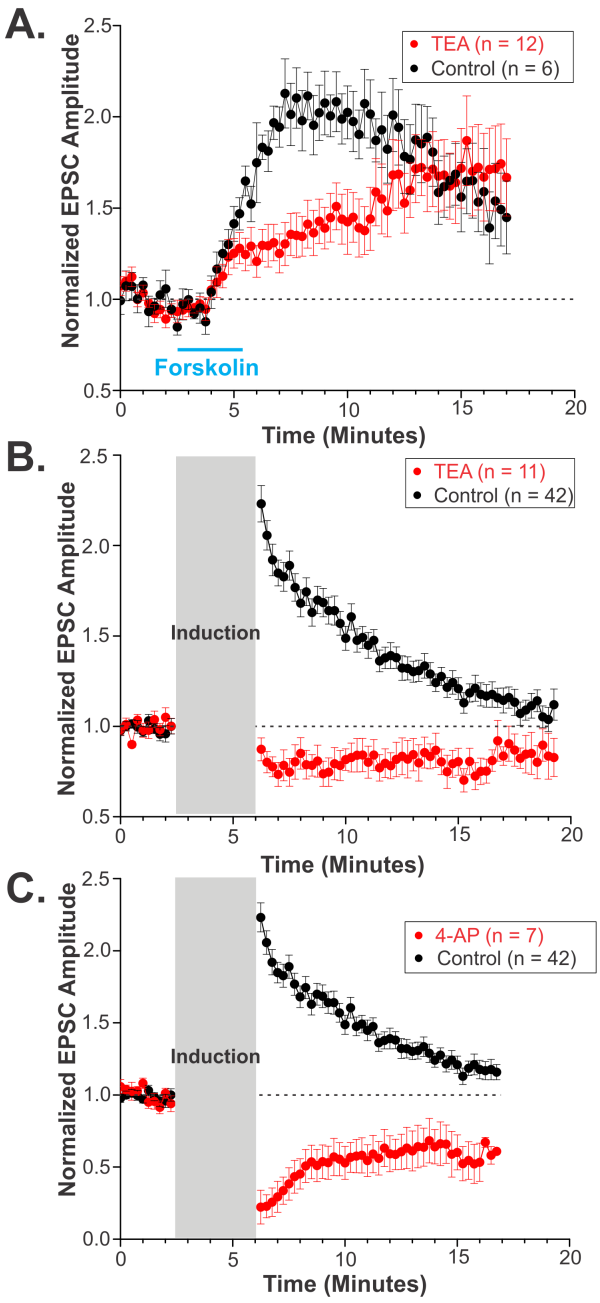

Figure 3.14. Short-term facilitation is attenuated by K⁺ channel blockers
A. The effects of the non-selective K⁺ channel blocker, tetraethylammonium chloride (TEA, 3 mM), were tested on forskolin-induced potentiation (10 μM; 3.75 minutes). TEA produced an attenuated potentiation of EPSC amplitude (n = 12), compared to control forskolin-induced potentiation ($p < 0.0001$). **B.** Next, tetanic stimulation in the presence of TEA was tested. The population data show that tetanic stimulation in TEA significantly reduced tetanus-induced facilitation compared to control conditions (n = 11; $p < 0.0001$). **C.** The voltage-gated K⁺ channel blocker, 4-AP (100 μM), was next tested with tetanic stimulation. In the presence of 4-AP, facilitation was completely blocked compared to control conditions (n = 7; $p < 0.0001$), and suppression of EPSC amplitude was observed, which lasted for the remainder of the experiment.

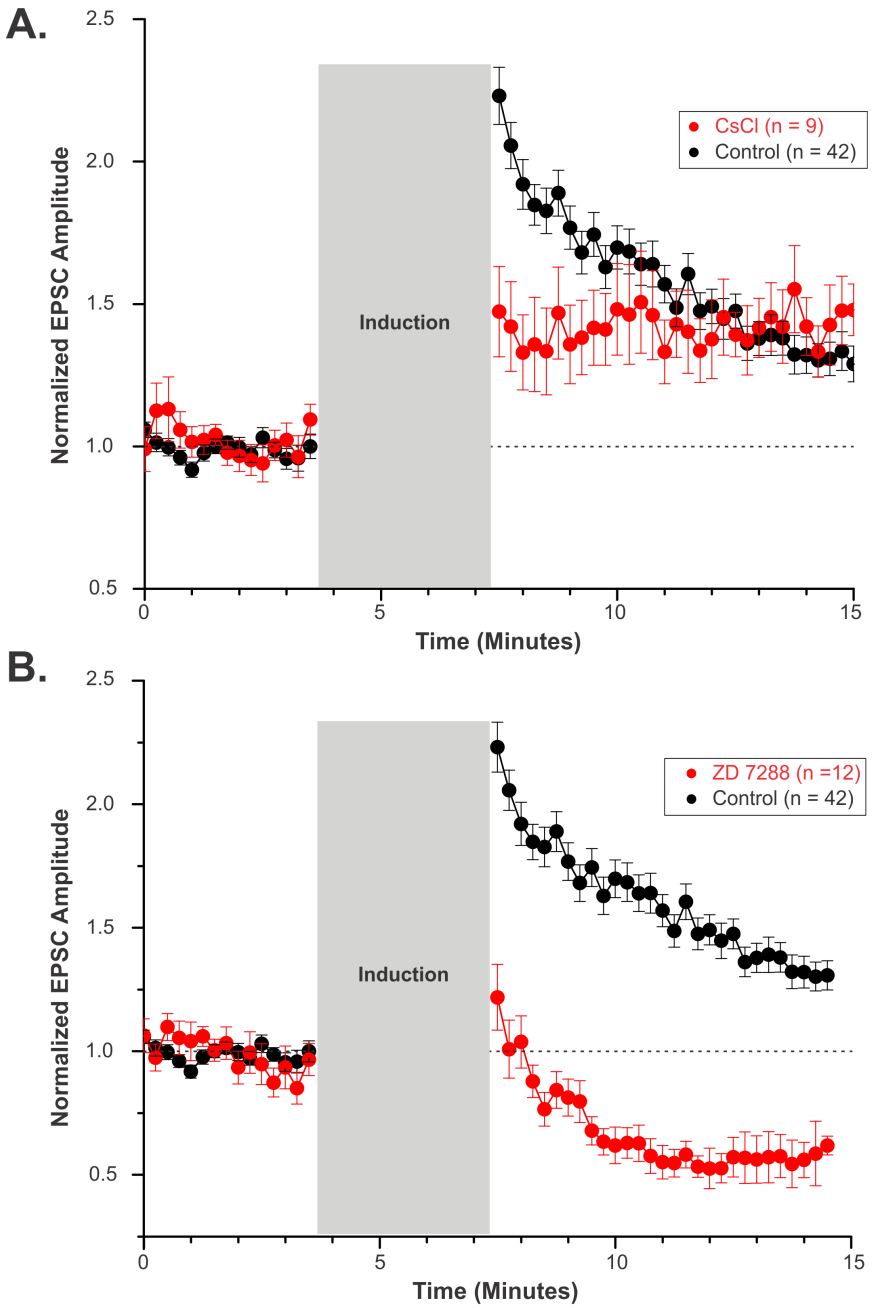

Figure 3.15. Short-term facilitation is mediated by I_h
A. Tetanic stimulation in the presence of cesium chloride (CsCl; 3 mM), a general I_h blocker, caused facilitation that was significantly reduced, compared to control conditions (n = 9; $p < 0.0001$). **B.** Next, tetanic stimulation in the presence of ZD 7288, a specific Ih blocker, was tested. In the presence of ZD 7288 facilitation was completely blocked (n = 12; $p < 0.0001$), and suppression of EPSC amplitude was observed.

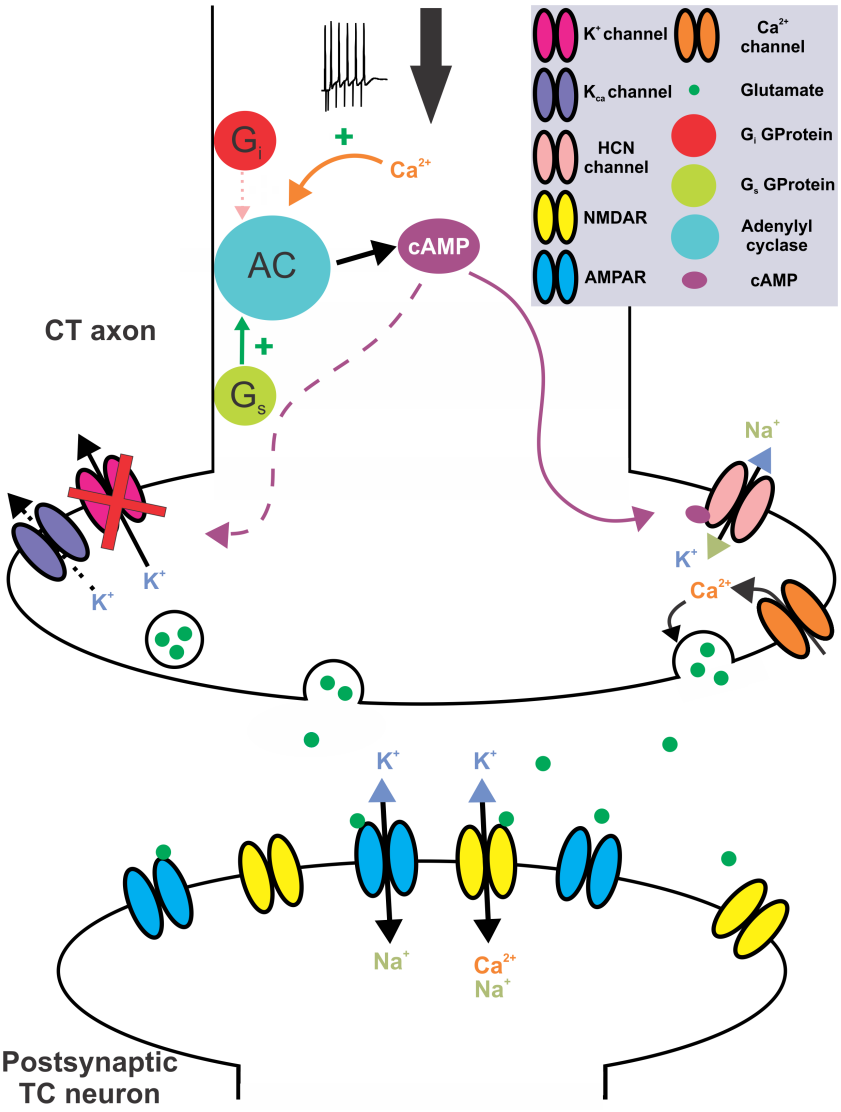

Figure 3.16. Schematic of proposed mechanism of action for tetanus-induced plasticity

The schematic shows a presynaptic corticothalamic terminal (CT axon) and postsynaptic thalamocortical (TC) neuron. Tetanic stimulation of corticothalamic afferents is likely producing elevated intracellular Ca^{2+} levels in the presynaptic corticothalamic neuron. We show that tetanus-induced facilitation is Ca^{2+}-dependent and adenylyl cyclase-dependent, so Ca^{2+} could activate adenylyl cyclase directly. We show that G-proteins mediate short-term facilitation, so $G\alpha_s$ could also be directly activating adenylyl cyclase. The contribution of $G\alpha_{i/o}$ is not fully understood, but it could potentially be modulating the duration of plasticity. Adenylyl cyclase activation leads to downstream production of cAMP. We hypothesize that cAMP can directly bind to HCN channels, inducing an inward current, which can depolarize the axon terminal. cAMP could also be indirectly causing the closure of K^+ channels, also leading to depolarization of the axon terminal. Depolarization of the terminal will lead to increased neurotransmitter release, which is likely responsible for short-term facilitation. The contribution of specific K^+ channels, such as Ca^{2+}-activated K^+ channels, is still unknown.

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CHAPTER IV: PLASTICITY OF INHIBITION IN THALAMOCORTICAL NEURONS

4.1 Introduction

GABAergic inhibition in the thalamus can regulate excitation within the thalamocortical circuit and mediate information flow to the cortex. Thalamocortical relay neurons receive GABAergic inhibition from the thalamic reticular nucleus (TRN) and local interneurons (Scheibel and Scheibel, 1966; Jones, 1985; Yen et al., 1985; Sherman and Guillery, 2006). While corticothalamic neurons project to thalamic relay nuclei, they also send efferent collaterals to TRN neurons. Considering efferents of TRN neurons in turn project to thalamocortical neurons, TRN neurons could play a role in modulating corticothalamic control of relay neuron output, potentially regulating the information that is transferred to the cortex (McAlonan et al., 2008). Since it is evident that the TRN can modulate relay neuron activity by providing GABAergic inhibition, we sought to determine the contribution of inhibitory plasticity at GABAergic synapses from the TRN to relay neurons.

My previous studies in Chapter II have shown short-term synaptic plasticity at excitatory synapses following tetanic stimulation of corticothalamic afferents. We showed an increase in excitation to relay neurons, so we wanted to determine if GABAergic synapses also display plasticity, and if these effects simultaneously occur to enhance thalamic throughput to the cortex. Recognizing the influence of inhibitory plasticity will help to understand if inhibition can be dynamically regulated, and how this may impact thalamic information transfer to the cortex.

Previous findings have described plasticity at other inhibitory synapses in the thalamus, dependent on NO activation (Sieber et al., 2013; Bright and Brickley, 2008), and

these findings will be important when determining the role of inhibitory plasticity in the thalamocortical circuit. Sieber et al. (2013) described long-term plasticity at inhibitory synapses between the TRN and relay neurons of the posterior medial nucleus that depends on postsynaptic burst discharge alone. Another study demonstrated that postsynaptic action potential firing could lead to an increase in tonic inhibition (Bright and Brickley, 2008). We decided to look at the effects following tetanic stimulation of reticulogeniculate synapses (TRN to dLGN synapses). From these findings, we can determine if tetanic stimulation of TRN afferents is sufficient to produce alterations in GABAergic transmission (Cox et al., 1998; Cox and Sherman, 2000; Govindaiah and Cox, 2004; Govindaiah and Cox, 2006).

In these experiments, we demonstrate synaptic plasticity at GABAergic synapses from the TRN to dLGN relay neurons. Plasticity of inhibitory currents could implicate a mechanism by which the TRN can dampen thalamic throughput following enhanced cortical feedback.

4.2 Materials and Methods

All experimental procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Michigan State University Institutional Animal Care and Use Committee.

Thalamic brain slices were prepared from Sprague Dawley rats (postnatal age: 15-25 days) of either sex were used in this study. Briefly, animals were deeply anesthetized with isofluorane (2-4% inhalation), perfused with cold, oxygenated slicing solution, and quickly decapitated. Brains were quickly removed and placed in cold (4°C), oxygenated (5% CO_2, 95% O_2) slicing solution containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgSO_4$,

0.5 $CaCl_2$, 26.0 $NaHCO_3$, 10.0 glucose, and 234.0 sucrose. Thalamic slices (300-400 μm) were cut in the parasagittal plane for dorsal lateral geniculate nucleus (dLGN) recordings similar to (Turner and Salt, 1998) using a vibrating tissue slicer. Slices were immediately transferred to a holding chamber with oxygenated physiological saline containing (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose. After 20-30 min, the holding chamber was reduced from 35°C to room temperature (~25°C), and slices were incubated for an additional 60 minutes before recording.

For recording, individual slices were transferred to a recording chamber maintained at 30°C with oxygenated (5% CO_2, 95% O_2) physiological saline (2.5-3 ml/min). Individual relay neurons were identified using a microscope equipped with differential interference contrast optics and 63x water-immersion objective (Zeiss Axioexaminer.A1). Recording pipettes were made from 1.5 mm OD borosilicate glass and had a tip resistance of 2-6 MΩ when filled with the following solution (in mM): 117 K-gluconate, 13.0 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2-ATP, and 0.4 Na-GTP, (pH 7.3, 290 mOsm). The internal solution resulted in a junction potential of ~10 mV and was corrected for in all voltage recordings. During recordings, pipette capacitance was neutralized, and the access resistance was continually monitored. Recordings typically had access resistances <20 MΩ. In addition, input resistance and holding current were continually monitored throughout the experiment.

Recordings were obtained using a Multiclamp 700B amplifier (Molecular Devices). Voltage-clamp recordings were collected with a holding potential of -60 mV. Inhibitory postsynaptic currents (IPSCs) were evoked by electrical stimulation (duration: 100 μs, intensity: 50-500 μA) of TRN efferents by placing a bipolar tungsten stimulating electrode

in the TRN (**Fig. 4.1A**). All experiments were done in the presence of non-NMDA and NMDA receptor antagonists, 6,7-Dinitroquinoxaline-2,3-dione (DNQX; 20 μM) and (3-((*R*)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid) (R-CPP; 10 μM) respectively, to pharmacologically isolate IPSCs. All drugs were purchased from Tocris (Minneapolis, MN). Data acquisition and analyses were performed using pCLAMP software (Molecular Devices, Sunnyvale, CA). Data were digitized at 10kHz, filtered online at 2.5 kHz, and stored on a computer. IPSC amplitudes were compared before and after tetanic stimulation, and were classified into groups that displayed either facilitation or suppression if amplitudes exceeded 2 standard deviation (SD) change from baseline IPSC amplitude that persisted >10 consecutive responses (2.5 min). After grouping cells into different categories based on synaptic responsiveness, cells were compiled and normalized to compare across cells. Peak changes from baseline levels were taken using an average of 5 consecutive responses from peak amplitude. Comparisons based on magnitude across stimulation groups were made using a Student's *t* test (unpaired). Data are presented as Mean ± SEM, and statistical significance was assessed with p value < 0.05.

4.3 Results

For these experiments, whole-cell intracellular recordings were obtained from 61 thalamocortical relay neurons.

A. Thalamocortical relay neurons display lasting potentiation following tetanic stimulation of GABAergic afferents

We first tested the hypothesis that plasticity of inhibition could be induced at reticulogeniculate synapses. To obtain synaptic recordings, the TRN was electrically stimulated while recording from dLGN relay neurons (**Fig. 4.1A**). IPSCs were

pharmacologically isolated with non-NMDA and NMDA receptor antagonists, continually present throughout experiment (Methods). After a stable IPSC amplitude was obtained, tetanic stimulation was applied. The tetanic protocol consisted of a 5-pulse train (100 Hz), repeated 400 times at 2 Hz (**Fig. 4.1B**). Following tetanic stimulation, we observed a variety of alterations in IPSC amplitude. In one group, the IPSC amplitude was facilitated in 16 of 51 (31%) neurons tested, and the IPSC amplitude returned to baseline levels within 8-10 minutes (**Fig. 4.2A**). The magnitude of initial facilitation was 76.0 ± 11.3%, which recovered over time. In another group of neurons (12 of 51: 24%), the tetanic stimulation produced a long-lasting facilitation of the IPSC amplitude, which did not recover during the posttetanic period (25 minutes). The magnitude of potentiation observed in these cells was similar to that seen in short-term facilitation (70.0 ± 20.3%) with the exception of the recovery (**Fig. 4.2B**). In another subpopulation of neurons (18 of 51: 35%), tetanic stimulation produced an initial suppression in IPSC amplitude (65.1 ± 7.4% decrease) that lasted 5 minutes and was followed by a long-lasting facilitation (62.6 ± 17.9% increase) that did not recover through the duration of the recording (**Fig. 4.2C**). In the remaining five cells, the IPSCs were unaltered by tetanic stimulation (**Fig. 4.2D**).

Since different forms of potentiation were observed, we determined if altering the stimulation parameters could affect the magnitude or duration of facilitation at GABAergic synapses. For the next series of experiments, we determined if the frequency of tetanic stimulation influences the magnitude of plasticity. In the control stimulation protocol, 5 pulses were given at a frequency of 100 Hz. As a comparison, all groups of responses (from **Fig. 4.2**) were combined to form the 100 Hz stimulation group. We tested the effect of 50 Hz tetanic stimulation, which produced a significantly greater magnitude of potentiation,

displaying an 87.7 ± 31.8% increase from baseline, compared to the 42.7 ± 10.6% increase from the group receiving 100 Hz stimulation (**Fig. 4.3**; n = 10; $p = 0.001$; unpaired t test). Together, these results suggest that the stimulation frequency can affect the response to tetanic stimulation at GABAergic synapses.

4.4 Discussion

Plasticity of inhibition in thalamic circuits and altered GABAergic inhibition on relay neurons can act as a means by which TRN neurons can dynamically regulate relay neuron activity. Altered inhibition can regulate information transfer through thalamocortical circuits, and these findings could lead to more insight into the TRN's role in potentially filtering out less salient information, prior to sending it to the cortex for perception. In this study, we demonstrated that long-term potentiation can be induced at GABAergic synapses in thalamic relay neurons following tetanic stimulation. Long-term plasticity at inhibitory synapses in thalamocortical circuits can have implications in altering thalamic throughput to the cortex by promoting long-lasting inhibition, and even dampening the transfer of information from relay neurons to the cortex. The TRN has been hypothesized to modulate information transfer by selectively silencing certain neurons, or enhancing cortical activation by inducing burst firing mode in relay neurons, effectively producing a larger cortical response (Crick, 1984; Sherman, 2001; Swadlow and Gusev, 2001). Long-term changes in GABAergic neurotransmission could affect information transfer for long periods of time, effectively modulating thalamic throughput based on environmental demands. For example, we have already shown that stimulation of corticothalamic afferents can cause short-term facilitation of excitatory currents in relay neurons, and primarily depression in TRN neurons (**Chapter 2**). Our findings regarding inhibitory plasticity can now integrate

the role of enhanced GABAergic transmission from the TRN. Stimulation of corticothalamic afferents produces enhanced excitation in relay nuclei and depression in TRN neurons. We show that stimulation of the TRN can lead to increased, and long-lasting inhibition onto relay neurons. So the differential effects of corticothalamic feedback (enhanced excitation in relay neurons, suppressed excitation in TRN), may be a mechanism to reduce TRN-mediated inhibition onto relay neurons, and thereby increase the overall information flow from thalamocortical neurons to the cortex.

The sustained inhibition in relay neurons could act as a method of gating sensory information transfer to the cortex, as suggested by Crick (1984) in his *searchlight hypothesis*. It is possible that the TRN selectively inhibits certain sectors of relay neurons, thereby impacting how information is transferred subsequently to the cortex. If an increase in inhibitory tone acts to silence certain relay neurons, while enhancing burst firing in other neurons, this could selectively increase the output of specific sectors of relay neurons. This selective silencing can also act to quiet surrounding noise, which may enhance signal transfer to the cortex.

This theory is especially interesting regarding our findings that show different forms of inhibitory plasticity following tetanic stimulation. While the majority of synapses display LTP of inhibitory transmission, there are some synapses that show short-term or unaltered IPSC amplitude following tetanic stimulation. These different forms of inhibitory plasticity could lead future studies regarding possible intrinsic differences in thalamic neurons that may be impacting plasticity following stimulation. . Since the largest groups of responses display short-term or long-term plasticity following tetanic stimulation, we could determine if there are any distinguishing factors in these inhibitory currents. For

example, there could be a correlation between the strength of inhibition onto thalamic relay neurons, and the form of plasticity that results after tetanic stimulation.

In a previous study, the experimenters showed that coincident pre- and postsynaptic stimulation of thalamoreticular afferents induced LTP in the excitatory response of TRN neurons (Astori and Luthi, 2013). We show that increased excitation in TRN neurons would lead to enhanced inhibitory output. Therefore, these findings could be combined to suggest that an increase in relay neuron activity causes long-term excitement in TRN neurons, leading to enhanced TRN-mediated inhibition at relay neurons, that we observed in our experiments. This could act as a feedback mechanism for the TRN to effectively control relay neuron output.

Another factor to consider when looking at plasticity at inhibitory synapses is the contribution from local interneurons. Aside from relay neurons, the dLGN contains local GABAergic interneurons, which also provide inhibition to relay neurons (Sanchez-Vives et al., 1996; Cox et al., 2003; Bickford et al., 2008). While our experiments are focusing on GABAergic output from the TRN to relay neurons, future experiments may include looking at the effects of TRN output onto interneurons. The TRN could be acting to primarily quiet local interneurons and specific sectors of relay neurons, which could act to increase the effect of incoming excitation on other groups of relay neurons. To test this theory, it is impetrative to replicate these experiments in a thalamic relay nucleus that is devoid of interneurons, such as the ventrobasal nucleus. We can determine if the same magnitude and groups of inhibitory plasticity are observed in the absence of any interneuronal influence. Additionally, we can test the contribution of GABAergic input onto interneurons, by stimulating the GABAergic afferents, as was done in the current experiments, and

recording from interneurons. A difference in synaptic efficacy in relay neurons compared to interneurons would help us gain insight into whether this plasticity is synapse specific, or if these effects are working together to specifically inhibit relay neurons.

Additionally, we showed that long-lasting forms of potentiation were predominant following tetanic stimulation. One form of lasting potentiation that was observed was similar to classical LTP, which demonstrates initial potentiation following the stimulation protocol, which plateaus and persists at a potentiated state. The other form of potentiation is more intriguing, since it shows an initial depression, followed by facilitation, and was observed in about 1/3 of the neurons. It is possible that tetanic stimulation induces synaptic depletion at the presynaptic terminal, but replenishment of more vesicles could occur over time, leading to the induction of lasting potentiation of IPSC amplitude after depression. Previous studies showed how retrograde messengers could affect presynaptic inhibitory transmission (Bright and Brickley, 2008; Sieber et al., 2013). It is possible that initial decay of the IPSC occurs due to presynaptic vesicular depletion, and potentiation persists following depression, due to postsynaptic release of NO, which has been shown to potentiate presynaptic release (Yang and Cox, 2007). It has also been shown that NO can directly depolarize TRN neurons robustly (Yang and Cox, 2008), which could be responsible for the late-onset, yet robust increase in inhibitory transmission. Future experiments addressing this question would involve observing the change in IPSC amplitude following wash in of a NO donor. We could then determine if NO-dependent potentiation can be occluded following tetanic stimulation. If stimulation does not cause further facilitation in the presence of NO, this would allow us to infer that tetanic stimulation is activating the NO pathway.

Next, we saw a greater magnitude of potentiation following 50 Hz tetanic stimulation, compared to 100 Hz stimulation. These findings suggest that there could be a frequency-dependent effect to plasticity following tetanic stimulation. Future experiments would include testing several stimulation frequencies to determine an optimal frequency that can induce reliable potentiation. It is possible that the high-frequency 100 Hz stimulation is depleting GABAergic synapses, leading to the biphasic LTP effect, or even suppressing the magnitude of the peak amplitude of LTP. More studies that test different frequencies can lead to further information about the effect of plasticity at different physiological firing frequencies, and what parameters may contribute to maximum potentiation.

Together, these findings show the effects of tetanic stimulation of GABAergic afferents onto dLGN relay neurons. We showed that LTP is reliably induced following tetanic stimulation, and that it can be observed in 59% of cells. There are several other responses that are evident, and they may each have a different role in transmitting information to the cortex. While we have preliminary data suggesting that the number of repetitions and frequency of stimulation can alter the magnitude of potentiation, these findings can lead to future experiments that investigate the differential responses following physiological firing frequencies.

An increase in inhibition from the TRN could act to modulate excitatory information transfer to the cortex. Long-term plasticity of inhibition could be a mechanism through which the TRN is involved in gating sensory information and transferring more salient information to the cortex.

APPENDIX

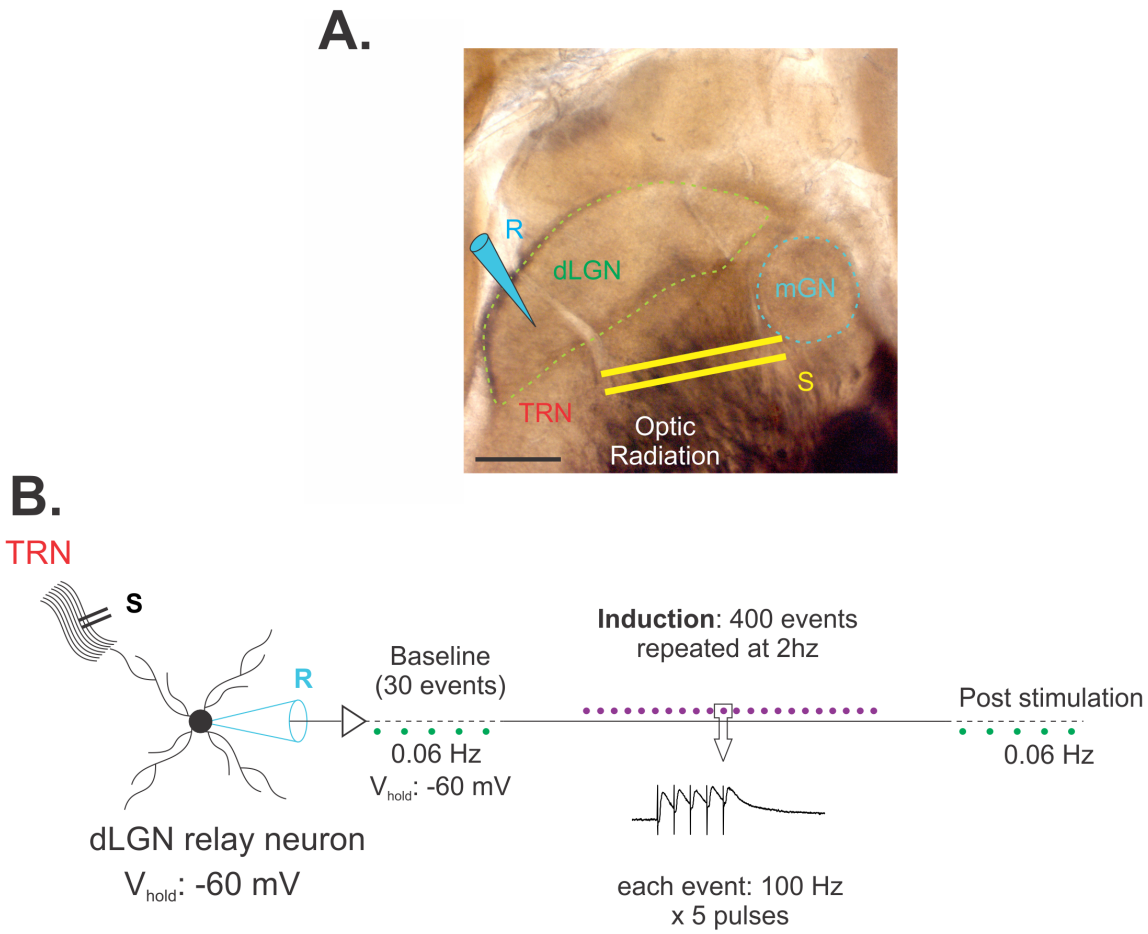

Figure 4.1. Brain slice and induction paradigm used to produce tetanus-induced plasticity at reticulo-geniculate synapses
A. Image of a parasagittal thalamic slice indicating placement of stimulating electrode (S) and recording electrode (R). Scale bar = 500 µm. **B.** Tetanic stimulation paradigm: IPSCs were evoked (V_{hold}: -60 mV) at 15-second intervals (green dots). After obtaining stable amplitude IPSCs for at least 30 consecutive responses, tetanic stimulation was delivered. Tetanic stimulation consisted of short trains (5 pulses at 100 Hz) repeated 400 times at 2 Hz (purple dots). Following the induction protocol, IPSCs were evoked at 15-second intervals.

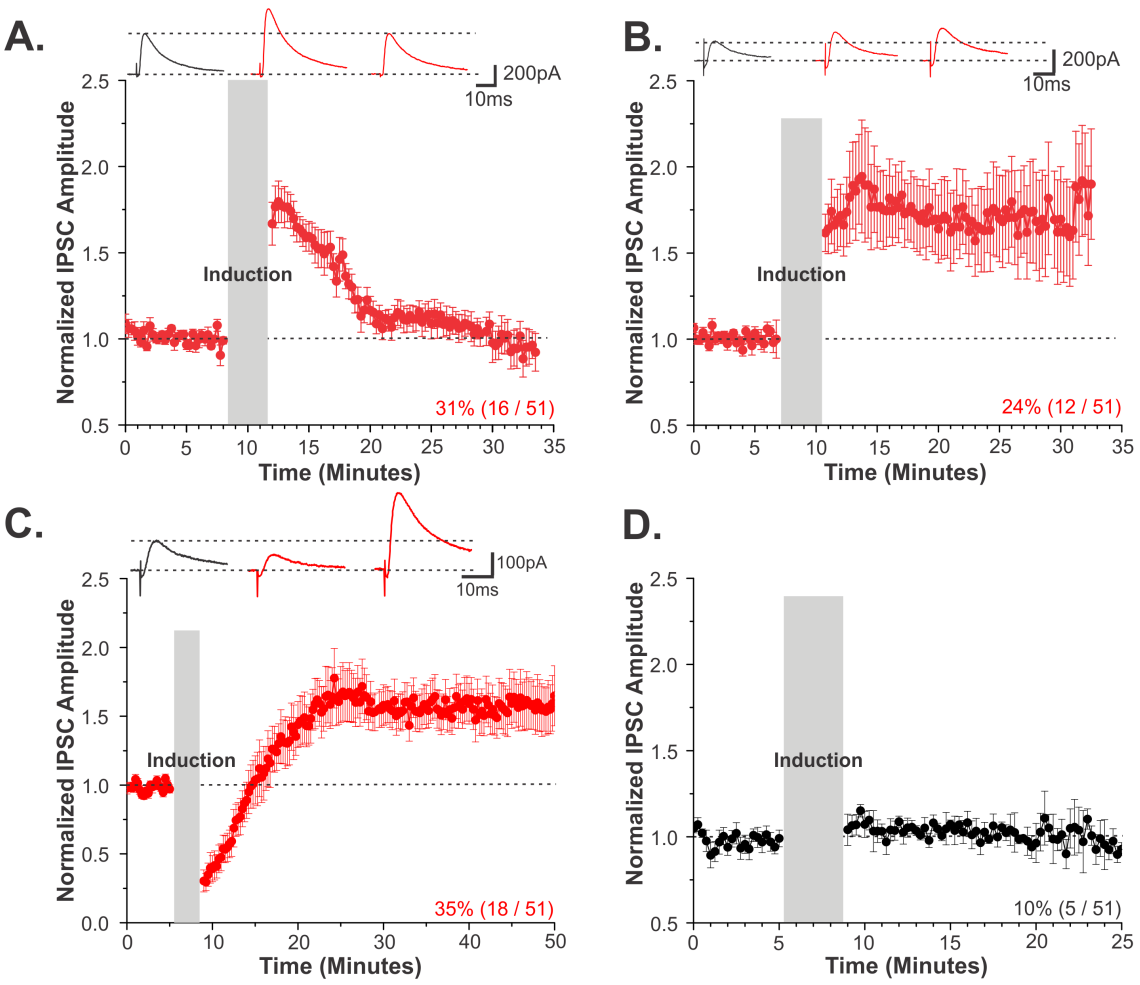

Figure 4.2. Tetanic stimulation produced multiple forms of facilitation of IPSCs
A. In a subset of neurons (16 of 51: 31%), tetanic stimulation produced facilitation of IPSC amplitude that returned to baseline levels within 15 minutes. *Top*, representative IPSCs (average of 5 consecutive responses) before and at 2 time periods (2 and 10 minutes post induction) following induction. **B.** In a different subset of neurons (12 of 51: 24%), tetanic stimulation produced long-lasting facilitation of the IPSC amplitude that did not recover for the duration of the recording. **C.** In 18 of 51 (35%) cells, tetanic stimulation led to an early suppression followed by a long-lasting facilitation of the IPSC amplitude. **D.** A small subset of neurons showed unaltered IPSC amplitude following stimulation.

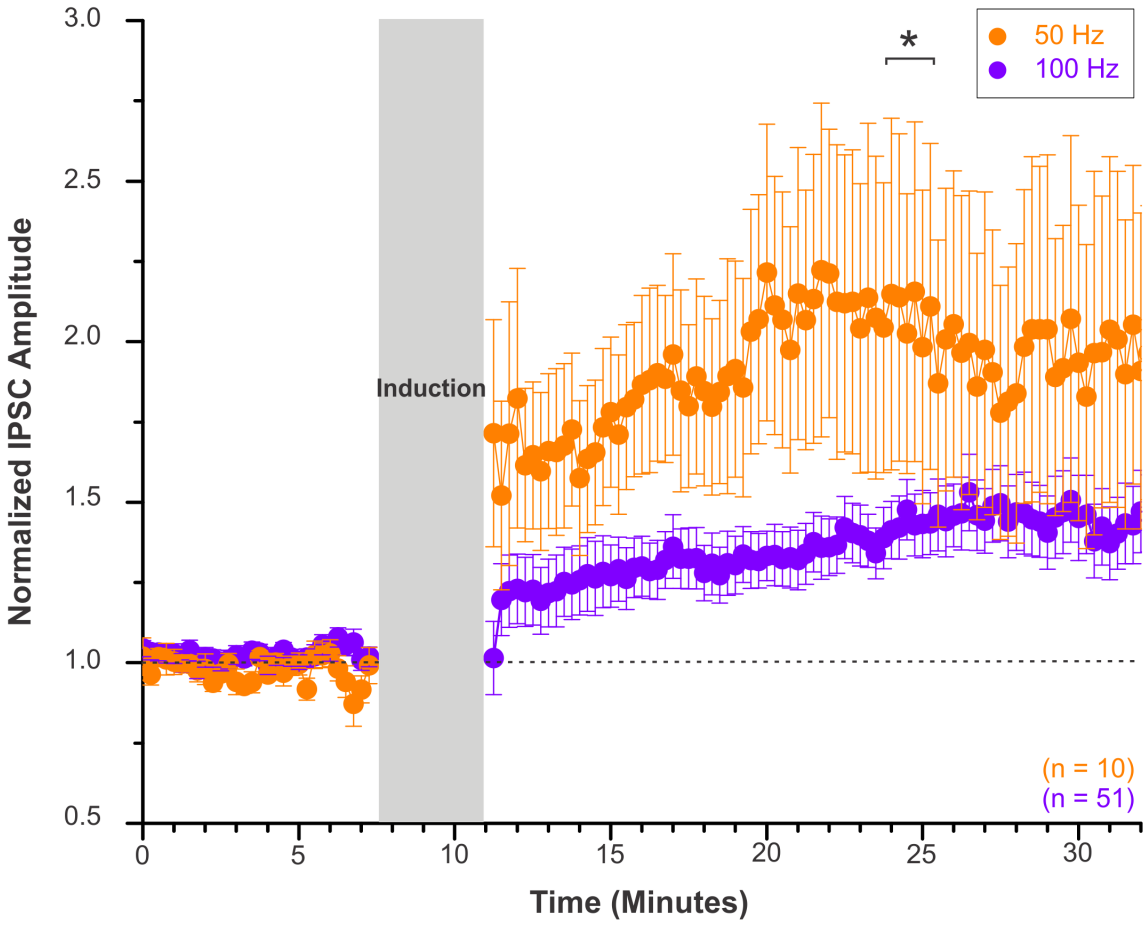

Figure 4.3. Frequency of tetanic stimulation affects the magnitude of facilitation
The effect of 50 Hz and 100 Hz tetanic stimulation (5 pulses, repeated 400 times at 2 Hz) on IPSC amplitude were compared. 50 Hz stimulation produced a greater magnitude of peak facilitation compared to the 100 Hz control conditions (n = 10; p = 0.001)

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CHAPTER V: GENERAL CONCLUSIONS

Through the experiments that I have detailed in my dissertation, I demonstrated the contribution of synaptic plasticity at different synapses in the thalamocortical circuit. From there, the underlying mechanism of tetanus-induced short-term plasticity was elucidated at corticothalamic synapses.

First, we showed state-dependent synaptic plasticity in thalamocortical relay neurons following burst or tonic discharge paired with a single EPSP from corticothalamic afferent stimulation. We show that paired burst stimulation produced a large percentage of cells that displayed short-term facilitation or depression, while paired tonic firing primarily produced depression of EPSC amplitudes. When tetanic stimulation was used instead of single EPSPs in the paired protocols, both stimulation paradigms produced short-term facilitation, irrespective of firing mode.

We hypothesize that the state-dependent alterations in synaptic activity observed following paired stimulation with a single EPSP could have a functional significance based on thalamic firing mode. Since tonic discharge is the predominant firing mode of neurons during the awake or aroused condition, it is possible that dampening of corticothalamic feedback occurs to facilitate faithful transmission of sensory information through the circuit. Tonic mode is hypothesized to be the main firing mode during waking, since it represents a linear transfer of information to the cortex, however, more spontaneous noise is evident during tonic firing, compared to burst firing (Guido et al., 1995; Wolfart et al., 2005). By suppressing corticothalamic feedback or excessive noise, this can allow the relay neuron to transfer information, with less excitatory interference. This could represent a

mechanism for optimized information flow through the thalamus in awake or attentive states.

In contrast to the paired tonic responses, short-term facilitation and depression are observed following paired burst stimulation. Rhythmic bursting is evident during sleep or drowsiness, so enhanced corticothalamic feedback may be one mechanism through which the cortex can enhance thalamic drive through the circuit during these states. Corticothalamic feedback can enhance throughput in several potential ways. It can simply produce a larger excitatory response onto thalamocortical dendrites, which will increase the excitability of the neuron, and enhance information transmission. Corticothalamic feedback could also directly enhance the sensory signal that is synapsing onto the relay neuron. It is known that corticothalamic synapses are primarily located on distal dendrites (Liu et al., 1995; Godwin et al., 1996; Erisir et al., 1997a; Van Horn et al., 2000). However, there could be some, albeit fewer, corticothalamic contacts on the proximal segments of the dendrites. Since retinal and other primary sensory afferent contacts are localized to proximal dendrites, then plasticity of corticothalamic synaptic efficacy could directly modulate the amount of sensory information that is entering the thalamus. One other possibility is that corticothalamic afferents can change the state of the thalamocortical neuron to affect information transfer. We know that corticothalamic influence can modulate thalamic firing mode (McCormick and von Krosigk, 1992). By causing enhanced excitatory feedback to a neuron in burst mode, it could have the potential to switch it to tonic firing mode, which would lead to sustained information flow to the cortex.

While corticothalamic afferents can modulate firing mode, it is important to note that in our experiments, a metabotropic glutamate response was not evident during the

stimulation protocols. While we locally stimulated corticothalamic afferents, in the intact circuit, there is likely much more corticothalamic influence, which could be sufficient to switch the firing mode of the neuron. We know that repetitive high-frequency stimulation of corticothalamic afferents is required to activate mGluRs (McCormick and von Krosigk, 1992), so it would be interesting to replicate these paired and unpaired experiments, while activating mGluRs. Through mGluR activation, we can see if plasticity is altered.

The different firing modes are mediated by different membrane conductances, which could contribute to the effects that were observed. We know that both burst and tonic discharge can produce backpropagating action potentials in thalamocortical neurons, through active dendritic conductances (Jahnsen and Llinas, 1984; Williams and Stuart, 2000). It is possible that when burst discharge was paired with an EPSP, a greater postsynaptic Ca^{2+} transient was generated at the dendrites, to produce a supralinear Ca^{2+} response, contributing to more cells showing facilitation. Tonic discharge, however, does not produce as large of a dendritic response (Zhou et al., 1997; Sieber et al., 2013). Since tonic firing primarily activates high-threshold activated Ca^{2+} channels, while burst firing can activate both high- and low-threshold activated channels, burst firing is more likely to produce a larger distal dendritic Ca^{2+} transient. Relay neurons have also been shown to display greater I_T activation at distal dendrites (Crandall et al., 2010; Errington et al., 2010), where corticothalamic afferents primarily synapse. Therefore, since burst mode is more likely to generate a greater distal dendritic Ca^{2+} response, summation of Ca^{2+} to supralinear magnitudes could be the factor that produces more facilitation in these neurons. While we saw a state-dependent effect with action potential firing paired with a single EPSP, this difference was completely abolished with the addition of the train. It is possible that

addition of the train produces a supralinear dendritic Ca^{2+} response in all neurons, irrespective of firing mode, leading to these effects.

We hypothesize that Ca^{2+} transients are contributing to the different plasticity responses, but several proposed experiments could allow us to directly investigate this hypothesis . We could verify that Ca^{2+} transients are produced at different magnitudes depending on the stimulation paradigm, by using the Ca^{2+} imaging technique. To test this, we could patch a corticothalamic neuron with a recording pipette containing both Alexa-Fluor dye and a Ca^{2+}-sensitive fluor-4 dye. 2PLSM coupled with Ca^{2+} imaging could be used to examine Ca^{2+} dynamics at the axon terminal of the corticothalamic cell. Following tetanic stimulation, we can see if there is an increase in Ca^{2+} levels, and if this increase persists for a time frame that is similar to that of the observed short-term plasticity. This can allow us to make several conclusions: if Ca^{2+} persists for a similar time duration, then we can conclude that Ca^{2+} levels are responsible for plasticity; if the Ca^{2+} levels decay, then there could be activation of other intracellular molecules, responsible for the longer-lasting effects. This reasoning is analogous to the ability of CaMKII to autophosphorylate itself, or alter channel conductance in early phase LTP, producing a lasting effect even after Ca^{2+} levels subside. Another method of testing this hypothesis would involve filling a thalamocortical neuron with Ca^{2+}-sensitive dye (Fluor-4), and imaging the distal dendrites, while performing the paired burst or tonic protocols. This will allow us to observe the magnitude of Ca^{2+} transients following either stimulation protocol. These experiments will allow us to directly test the hypothesis that paired tonic stimulation evokes a smaller dendritic Ca^{2+} transient than paired bursting.

Another possibility to test the Ca^{2+} hypothesis involves using the transgenic GCaMP mouse (Chen et al., 2013). GCaMP is a Ca^{2+} sensor, which will fluoresce in response to Ca^{2+} influx. Through selective breeding, GCaMP can be expressed in layer 6 pyramidal neurons. We can then patch the postsynaptic relay neuron, and fill it with Fluor-4 to examine Ca^{2+} dynamics simultaneously in the pre- and postsynaptic neurons.

When performing the paired burst experiments, cells showed potentiation, suppression or no change in synaptic efficacy. However, these different groups of effects bring up an interesting question regarding the factor responsible for these different responses. It is possible that some cells may show potentiation if the burst adequately backpropagates into more dendrites, and shows low attenuation due to passive cable properties. Many factors may contribute to this, such as dendrite thickness/length, dendritic arborization, or strength of the burst (due to LTS size, number of action potentials, etc.). If we can examine the Ca^{2+} dynamics through Ca^{2+} imaging experiments, we may be able to "predict" the response of the relay neuron to paired burst stimulation, based on the size of the Ca^{2+} transient that innervates the dendrites. If there is no correlation in Ca^{2+} transients and synaptic efficacy following the stimulation paradigm, there may be some other contributing factor that is responsible for the different types of plasticity.

Another feature that could be examined when determining the cause of the different plasticity responses is the morphology of the relay neuron. Friedlander et al. (1981) showed morphological and physiological distinctions in relay neurons, and studies have shown similar categories of thalamic neurons in rodents, as well (Friedlander et al., 1981; Krahe et al., 2011). A potential experiment to test the contribution of different synaptic

morphologies involves filling a relay neuron with Alexa Fluor dye, and determining if there is any morphological correlate with the plasticity observed. Although Krahe et al. (2011) showed that there were no physiological differences in the rodent relay neuron categories, these proposed experiments could allow us to determine if morphological structure (dendritic arborization, thickness), could affect plasticity and the degree of backpropagation into the dendrites.

Through the course of these experiments, we have tried different variations to the stimulation paradigm to induce the greatest magnitude of plasticity. However, a systematic approach to determine which aspects of stimulation protocol can alter synaptic effects will be extremely informative in determining the physiological significance. Firstly, we want to determine if there is a precise correlate between timing and number of repetitions. A comprehensive analysis can be done, using different numbers of repetitions or different timings between the pre- and postsynaptic stimulation. One question that should be addressed is if spike-timing-dependent plasticity, which was shown previously in synapses of the thalamocortical circuit (Hsu et al., 2012), can be induced by using the paired stimulation paradigm. In line with those experiments, we want to determine if the timing between the two stimuli can affect plasticity. We did a small number of trials in the dLGN using three different time delays (5ms, 35ms, and 200ms), which did not produce any difference in the magnitude of plasticity, but a larger, more representative sample would allow us to make a better conclusion regarding timing.

In chapter 2, we showed that while the corticothalamic pathway could readily display short-term plasticity following repeated stimulation, the retinogeniculate synapses do not show plasticity with paired stimulation. These findings are extremely interesting,

since it shows how the corticothalamic pathway can preferentially induce plasticity. These findings were also confirmed by Hsu et al. (2010) in the medial lemniscal synapses to the VB (Hsu et al., 2010). As alluded to previously, these findings could have implications regarding the ability of corticothalamic afferents to affect thalamic throughput to the cortex following repeated stimulation, while the optic tract does not show alteration of information transfer. The circuit could be acting to transfer unaltered incoming sensory information, while corticothalamic feedback can affect how the information is sent to the cortex. Interestingly, high-frequency tetanic stimulation induced depression at retinogeniculate synapses, which was opposite to the response seen at corticothalamic synapses. It is possible that sensory transfer can be dampened if the stimulation exceeds the capabilities of the synapse. Since retinogeniculate synapses have a high probability of release, it is possible that they cannot sustain repetitive stimulation. Functionally, this could act as a compensatory mechanism for over excitation through the sensory afferents.

We have shown that the corticothalamic and retinogeniculate synapses show different forms of plasticity, following stimulation. We know that there is a differential distribution of corticothalamic versus sensory synapses along the relay neuron dendrite, with sensory synapses occurring proximally, and corticothalamic synapses localized more distally (Liu et al., 1995; Erisir et al., 1997a; Erisir et al., 1997b). Glutamate uncaging experiments can be done based on this localization, to determine whether the different plasticity effects can be induced on one dendrite. For these experiments, we could fill a relay neuron with Alexa Fluor dye to visualize it, and uncage glutamate at either proximal or distal locations while administering burst or tonic firing. Our previous uncaging experiments were all done at distal dendritic locations, so this will also allow us to

determine if glutamatergic binding at proximal dendrites can induce plasticity. These results will allow us to further confirm whether there is a postsynaptic effect of the distal or proximal synapses, or if the differences are solely due to presynaptic neurotransmitter release.

In chapter 2, we also showed that the TRN shows long-term depression following paired bursting and internal capsule activation. These results were interesting, since paired burst firing primarily produced depression. These results support our hypothesis that plasticity increases thalamic throughput, since suppression of EPSCs will reduce TRN-mediated inhibition onto relay neurons.

While our experiments involved stimulation of the internal capsule, which contains corticothalamic afferents, thalamocortical afferents are also present in this tract. It is difficult to determine the contribution of each afferent when stimulating the internal capsule, since electrical stimulation of either afferent in the internal capsule will produce a monosynaptic EPSC in the TRN neuron. Therefore, to accurately conclude that this effect is due to corticothalamic activation, it would be necessary to isolate the corticothalamic afferents. This can be done by transgenically expressing or injecting a channel rhodopsin (ChR2) virus (adeno-associated virus, pAAV2-CaMKIIa-hChR2 (H134R)-EYFP) into layer 6 pyramidal neurons, to allow for selective optogenetic stimulation of corticothalamic afferents. A light-activated EPSC can be produced in a TRN neuron by activating (470 nm) the corticothalamic terminals containing ChR2. Replicating the same stimulation paradigm using optogenetics will allow us to isolate corticothalamic afferents and confirm that short-term depression is due to corticothalamic activation.

We know that corticothalamic afferents send collaterals through the TRN, and understanding how plasticity affects both the corticogeniculate and corticoreticular synapses would help us form a comprehensive understanding of the circuit properties of plasticity. An interesting experimental manipulation would involve optogenetically stimulating a corticothalamic afferent that synapses onto a relay neuron and TRN, and record from both cells. We can determine if the opposite plasticity trends can be induced in both neurons. There are some difficulties that may hinder this experiment from being successful, such as slice connectivity (e.g. TRN, VB and corticothalamic terminals to both nuclei would have to be in the same slice; difficulty in locating thalamic cells that are synapsing to the same corticothalamic afferent), but findings from these experiments could yield informative results about how the TRN and VB respond differently to the same stimulation.

In Chapter 3, we determined the mechanism for activity-dependent short-term plasticity in VB relay neurons. We hypothesize that a reduction in K^+ channel conductance and an increase in I_h is responsible for the depolarization of corticothalamic terminals, which will lead to enhanced presynaptic neurotransmitter release. While we have determined that neurotransmitter release is increased, we can look further into this aspect by determining if there is a change in the size of the readily releasable pools of vesicles, which could be attributed to this increase in synaptic activity. Osmotic shock experiments could be performed, by applying an extracellular solution of hypertonic sucrose, which will cause a dramatic release of all readily releasable vesicles (Rosenmund and Stevens, 1996), to the slice. Osmotic shock experiments can be done at baseline (prior to stimulation), and then following tetanic stimulation, to determine if any change can be observed in the size of

the readily releasable pool. These results will allow us to determine how increased neurotransmitter release is mediated, following alterations in ion channel conductances.

Additionally, we have mechanistically showed that short-term facilitation is not resultant from protein kinase activation, which can lead to downstream transcriptional modifications. Many forms of hippocampal LTP and corticothalamic LTP require protein kinase activation, which contributes to the lasting effects (Lopez-Garcia et al., 1996; Castro-Alamancos and Calcagnotto, 1999; Nicoll and Malenka, 1999) by altering specific transcription factors. We show that short-term facilitation is not dependent on protein kinase activation, which may indicate that this form of plasticity can be dynamically regulated. LTP at corticothalamic synapses shows a persistent enhancement in information transfer through the thalamocortical circuit, but the functional significance of such long-lasting effects are not clear. We hypothesize that short-term plasticity can be a mechanism of transient and inducible regulation of corticothalamic output. It is transient, in that the effect recovers to baseline over 15-20 minutes; it is inducible, since changes at the terminal are likely not resultant from transcriptional modifications, so potentiation can rapidly be reversed based on the environmental situation.

Together, we can draw conclusions from our findings, to form a comprehensive hypothesis for the role of synaptic plasticity at various synapses in the circuit. We show that short-term facilitation is primarily observed at corticothalamic synapses, following tetanic stimulation. This will likely provide enhanced information flow to the cortex. We also show that corticothalamic afferent output is reduced to TRN neurons. A reduction in corticothalamic feedback will lead to a decrease in inhibition to relay neurons. This decreased inhibition will also lead to enhanced thalamic throughput to the cortex.

We show data suggesting that long-term potentiation of inhibitory transmission occurs following tetanic stimulation of TRN afferents. This would lead to decreased thalamocortical output; however, TRN activity would be decreased in our model (due to the suppression of corticothalamic output to TRN). Therefore, this enhanced inhibition might occur when there is over activity in the circuit, causing a high-frequency stimulation of the TRN. In this way, the TRN can be equipped to quiet excessive activity by providing long-term inhibition. Together, these mechanisms may work together to increase thalamic throughput to the cortex.

It is possible that the behavioral relevance of synaptic plasticity is to modulate the transfer of information during selective attention. While selective attention processes are thought to occur only in higher cortical areas, several studies have suggested that attention could occur in its earliest stages at the thalamic level (O'Connor et al., 2002; McAlonan et al., 2008; Ling et al., 2015). Studies in the LGN, show that feedback modulation allows the visual system to modify incoming feature-specific signals at its earliest processing site. In this way, corticothalamic feedback might selectively bolster visual field information in the LGN (Ling et al., 2015), to enhance the transmission of more salient information.

LGN recordings show that attention modulates visual signals at the thalamic level, before it even reaches the cortex. This occurs by increasing the responses of relay neurons, while decreasing the responses of the TRN (McAlonan et al., 2008). This interplay at the thalamic level, which has been shown in awake macaque monkeys, is implicated by our plasticity experiments. These studies give us further evidence that selective sectors pertaining to specific visual information may be differentially regulated based on attentive demand. In a series of fMRI experiments in humans, studies show that LGN activity is

enhanced during the attentive state, and suppressed when humans were ignoring a stimulus. Additionally, anticipating a visual stimulus caused an increase in baseline activity of the LGN (O'Connor et al., 2002). These findings are extremely interesting, since they may provide functional significance in terms of selective attention for the different synaptic responses that we see following stimulation. Corticothalamic afferents could be selectively enhancing synaptic activity onto relay neurons that are involved in processing a visual response to facilitate the transfer of more salient information, pertaining to attention. Simultaneously, corticothalamic afferents could be suppressing the activity of other neurons, which may be involved in irrelevant or background visual information transfer. The nature of this modulation can be affected by thalamic firing mode and intensity of corticothalamic feedback.

Together, these studies provide a functional correlate for plasticity in the thalamic circuit. We showed detailed experiments regarding plasticity and potential mechanisms within the thalamus at the cellular and synaptic level. From these findings, we can form conclusions about how plasticity at corticothalamic synapses may manifest in attention and behavioral states. Since the thalamus is the first stage at which top-down feedback can affect visual processing, our findings may provide evidence regarding how selective attention is modulated by synaptic plasticity throughout the thalamocortical circuit.

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