8-2020

Cellular And Circuit Properties Of Slow Oscillations In The Thalamic Reticular Nucleus

John O'Malley

CELLULAR AND CIRCUIT PROPERTIES OF SLOW OSCILLATIONS IN THE THALAMIC RETICULAR NUCLEUS

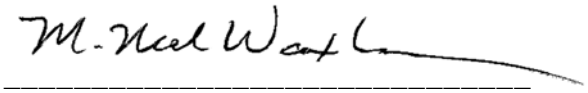
by

John Joseph O'Malley, M.A.

APPROVED:

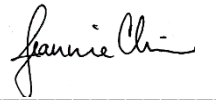
_____
Michael Beierlein, Ph.D.
Advisory Professor

_____
Ruth Heidelberger, M.D., Ph.D.



_____
M. Neal Waxham, Ph.D.

_____
Harel Z. Shouval, Ph.D.



_____
Jeannie Chin, Ph.D.

_____
Michael Lorenz, Ph.D.

APPROVED:

_____

Dean, The University of Texas

MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences

CELLULAR AND CIRCUIT PROPERTIES OF SLOW OSCILLATIONS IN THE THALAMIC RETICULAR NUCLEUS

A

DISSERTATION

Presented to the Faculty of

The University of Texas

MD Anderson Cancer Center UTHealth

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

by

John Joseph O'Malley, M.A.

Houston, Texas

July, 2020

To Michael and Kevin, *my brothers.*

Acknowledgments

As I reflect back on my PhD I realize how much truth there is to the idea of how we stand on the shoulders of giants. I know it's a bit cliché, but without those that came before me I would not have been able to complete my dissertation. So, there are several people I need to thank that have made this dissertation and PhD possible, and guided me along the way.

First and foremost, I have to thank Michael. No amount of words, written or spoken, can fully express my gratitude towards him. He is an amazing scientist and his scientific rigor has pushed me to be the best scientist I can be. I always enjoyed our discussions, over lunch and in lab, about my projects and science in general. I can only hope that I become half the scientist he is. In addition to learning how to be a good scientist from Michael, I have learned how to be a great mentor. Michael's passion for science goes beyond conducting it, he wants to help guide you along the way to achieve whatever goals you have for your career, whether it is in science or not. He always leaves his door open making it easy to approach him with any question, big or small. This policy is not just for his students in lab, but for anyone that stops by. He works to help any student that comes to him. He deeply cares about you as a person, wants to help you develop in to the best person and scientist you can be, going above and beyond to provide the means to achieve your goals. He has taught me so much and I will use what I have learned when I am mentoring my own students. I am more that grateful to be able to have been a part of his lab and that I can call Michael both my mentor and friend.

I also have to thank each and every member of my committee as they also shaped my career in science. Ruth, whose excitement for science, especially electrophysiology, helped me appreciate the little details about it. Jeannie, whose optimistic positivity always kept me going even when science was difficult, she was always excited to hear what I had done. Harel, who

always asks the tough questions, which forced me to think critically about my data and about what I say when talking about it and answering questions. Neal, who's critical and scientific thinking made me a better scientist and pushed me to think carefully about my project and the meaning of my data. Mike who presents science with such eloquence, describes data simply and clearly, has shown me what it takes to be a good communicator of science. I chose my committee in order to better myself as a scientist and I am very glad each of you could be a part of my PhD journey.

In addition to Michael and my committee, I want to thank my fellow lab members. Both Rajan Dasgupta and Fred Seibt who not only helped me when I first learned to record, but were also there for me as friends. I am eternally grateful to have them in my life and look forward to interacting with them in the future whether it be at conferences or just catching up over video chats or emails.

To the friends that I have made along the way, I am glad to have you in my life and I will cherish every memory with each of you. There are a few people that I would particularly like to thank as they helped in one way or another with this dissertation. To Sydney Moyer and Dan Campbell, whose friendship is invaluable. I will cherish what time we spent together, especially during the period of my writing, and I am excited to see where our friendship goes with our next steps in our lives. To Taylor Chrisikos and our friendship and especially our early morning bike rides. Those bike rides helped to clear my mind before writing, and lead to several scientific discussions both useful for this thesis and for fun. To Natalie Sirisaengtaksin and Katherine Savell for their selfless friendships, and for reading this and providing feedback. Lastly, to Iman Sahnoune and Jeanne Manalo for helping with my defense and their friendships throughout my PhD.

None of this would have been possible if not for my parents. It is because of them that I enjoy science. Mom is a teacher and her passion for teaching has inspired my thirst for knowledge. Fun fact she was my math teacher for two years, and if you think she gave me any favoritism, she wouldn't let me ask her questions at home. So I would have to get help from my Dad, and I'm sure hearing "that's not how Mom taught it" several times was not easy. My dad has also been a big influence for my love of science and data. I will always remember how he taught me abought plotting graphs at a very young age using the carpet to draw axis and gumballs to understand data visualization. To me the best part of science is seeing the plots change after entering in data and I know that is because of what my Dad did.

There are several other people I have to acknowledge who were there to guide me on my way before I was even a PhD student. Jeremy Day gave me the opportunity to be a lab technician in his lab as he was just starting it. I learned so much that year and his guidance has a lasting influence on my scientific career. David Daniels, who was not only the hottest professor in 2013 according to ratemyprofessor.com, but also went out of his way to guide me on this path, contacted Jeremy and spoke on my behalf. Both Jeff Dyche and Dan Holt who were amazing mentors throughout my master's degree. Jessica Irons for teaching me to teach and the reason I enjoy it so much. Lastly, Kevin Apple who was my undergrad advisor and my first PI. It was in his lab that I fell in love with the scientific process and knew I wanted to make a career out of it.

And to you, the reader. Whether you've come across this because I've shared this with you, you were interested in the TRN, or you somehow stumbled upon this while searching for articles, thank you for taking the time to look at this and hopefully you enjoy the read.

Cheers

CELLULAR AND CIRCUIT PROPERTIES OF SLOW OSCILLATIONS IN THE THALAMIC RETICULAR NUCLEUS

John Joseph O'Malley, M.A.

Advisory Professor: Michael Beierlein, Ph.D.

Abstract. During sleep, neurons in the thalamic reticular nucleus (TRN) generate distinct types of oscillatory activity. While the reciprocal synaptic circuits between TRN and sensory thalamic nuclei underlie the generation of sleep spindles, the mechanisms regulating slow (<1 Hz) forms of thalamic oscillations are poorly understood. Under *in vitro* conditions, in the absence of synaptic inputs, TRN neurons can generate slow oscillations in a cell-intrinsic manner. Activation of postsynaptic Group 1 metabotropic glutamate receptors (mGluR) leads to long-lasting plateau potentials thought to be mediated by both T-type calcium currents and calcium-activated nonselective cation currents (I_{CAN}). However, the identity of I_{CAN} and the possible contribution of thalamic circuits to slow rhythmic activity remain unclear. Using intracellular recordings of neurons in thalamic slices derived from adult male and female mice, I recorded slow forms of rhythmic activity in TRN neurons. Slow oscillations were driven by fast glutamatergic inputs from thalamic relay neurons, but did not require postsynaptic mGluR activation. For a significant minority of TRN neurons (33%), synaptic inputs or brief depolarizing current steps led to plateau potentials and persistent firing (PF), and in turn, resulted in persistent synaptic inhibition in postsynaptic relay neurons of the ventrobasal thalamus (VB). Pharmacological approaches indicated that plateau potentials were triggered by calcium influx through T-type calcium channels and mediated by calcium and voltage-dependent transient receptor potential

melastatin 4 (TRPM4) channels. Taken together, my results suggest that thalamic circuits can generate slow oscillatory activity, mediated by an interplay of TRN-VB synaptic circuits that generate rhythmicity and TRN cell-intrinsic mechanisms that control PF and oscillation frequency.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AD: Alzheimer's disease

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

CB: Calbindin

ChR2: Channelrhodopsin

CT: Corticothalamic

CTC: Corticothalamocortical loop

dLGN: Dorsal Lateral Geniculate Nucleus

EPSC: Excitatory Postsynaptic Current

GABA: γ-Aminobutyric Acid

GPCR: G-Protein Coupled Receptor

I_{CAN}: Calcium-Activated Nonselective Cation Current

I_{NAP}: Persistent Sodium Current

IPSC: Inhibitory Post Synaptic Current

LGN: Lateral Geniculate Nucleus

LP: Lateral Posterior

mAChR: Muscarinic Acetylcholine Receptor

mGluR: Metabotropic Glutamate Receptor

MGN: Medial Geniculate Nucleus

nAChR: Nicotinic Acetylcholine Receptor

NBQX: 2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline

PF: Persistent Firing

POm: Posteriomedial Thalamus

PV: Parvalbumin

SOM: Somatostatin

SWS: Slow Wave Sleep

TC: Thalamocortical

TRN: Thalamic Reticular Nucleus

TRPC: Canonical Transient Receptor Potential

TRPM: Melastatin Transient Receptor Potential

VB: Ventrobasal Thalamus

Chapter 1: Introduction

The thalamic reticular nucleus (TRN) is entirely GABAergic and positioned between the thalamus and the internal capsule. It regulates thalamocortical activity during sensory processing and participates in oscillatory activity observed during sleep. While several works have elucidated the mechanisms that underlie TRN function during sensory processing (Crick, 1984; Pinault, 2004), there is a lack of insight into how cell-intrinsic and circuit properties shape TRN oscillations. In this introduction, I will first discuss the overall structure and function of the TRN, describing the topographical and functional organization of TRN sectors. Next, I will outline the cell-intrinsic properties that shape both neuronal excitability and firing properties within the TRN, and explain how these properties shape TRN function. Lastly, I will describe how the circuits formed by TRN shape activity and function, and highlight unanswered questions.

1.1 Overall Structure and Function of the TRN

First identified at the end of the nineteenth century (Köliker, 1896), the TRN has become of interest due to its multifaceted role in shaping brain activity. The TRN is important for human cognition as it shapes sensory processing, attention, and memory formation (Raeva & Lukashev, 1993; Ross, Graham, & Adams, 1993; Viviano & Schneider, 2015; Manoach et al., 2016). Its shell-like structure and location deep within the brain limit how far we can probe the human TRN. Thus, researchers have turned to animal models in order to gain additional mechanistic insight. *In vivo* and *in vitro* studies in TRN from monkey (Zikopoulos & Barbas, 2012; Williamson et al., 1994), cat (Crabtree, 1996, 1998a; Steriade et al., 1993a; Steriade et al, 1993b), ferret (Kim & Mccormick, 1998) and rodent (Halassa et al., 2014; Lee et al., 2007; Huguenard & Prince, 1994) have led to a better understanding of its structure and function (Pinault, 2004). These animal studies corroborated findings using human subjects that implicate a role of the TRN in attention, suggesting that the TRN

works mainly to suppress irrelevant sensory information and narrows attention, similar to a searchlight (Crick, 1984). However, further studies show the TRN has additional functions beyond sensory processing (Halassa et al., 2011; Kim et al., 2012; Vantomme et al., 2019); moreover, given that the TRN has a complex structural organization (Pinault & Deschênes, 1998; Crabtree, 2018) and receives several different inputs that shape and modulate activity (Pinault, 2004; Lam & Sherman, 2011; Beierlein, 2014), the idea that the TRN acts as a uniform regulator has changed. In the following, I will first describe synaptic projections involving the TRN, its structural organization, and finally the general functions of the TRN.

1.1.1 Synaptic outputs and inputs of the TRN

TRN outputs. The manner by which the TRN shapes cognition and brain activity is determined in a large part by its projection targets. Golgi staining and juxtacellular anterograde labeling of TRN neurons show that the targets of axonal projections are found exclusively in various thalamic nuclei (Pinault, 1994; Pinault & Deschênes, 1998; Wang et al., 2001). Initially, TRN neurons were thought to only project to sensory thalamic nuclei; however, more recent evidence has shown that TRN projections also extend to the anterior (Halassa et al., 2014) and midline portions (Dong et al., 2019; J. H. Lee et al., 2019) of the thalamus.

The thalamic nuclei associated with sensory processing can be grouped broadly into two categories, thalamic relay (or first order nuclei) and higher order nuclei. First order thalamic nuclei receive sensory inputs and cortical feedback from layer VI cortical neurons in primary sensory cortices (Guillery & Harting, 2003). Several studies established that the TRN projects to the first order thalamic nuclei, the ventrobasal nucleus (VB), the dorsal lateral geniculate nucleus (dLGN), and the ventral portion of the medial geniculate nucleus (MGN). Injections of retrograde tracers into these nuclei show connectivity to the TRN (Crabtree, 1996, 1998). Moreover, stimulation of TRN in

thalamic slices evoke inhibitory postsynaptic currents (IPSC) in intracellular recordings from the VB (Zhang et al., 1997; Ulrich et al., 2018) and LGN (Guido, 2018), thereby confirming synaptic connectivity. Under *in vivo* conditions, optogenetic activation of TRN projections results in a pause of activity in first order thalamic cells, most likely due to inhibition (Halassa et al., 2011). Taken together, there is extensive work showing the TRN has inhibitory projections to the first order thalamic nuclei.

The higher order thalamic nuclei consist of the pulvinar complex, lateral posterior (LP), posteromedial nucleus (POm), and the dorsal medial geniculate nucleus (dMGN). These subregions differ from the first order nuclei in that they receive cortical feedback from both layer V and VI neurons located in both primary and secondary sensory areas (Crabtree, 2018). Images obtained from retrograde labeling and electron microscopy from the pulvinar complex (Fitzgibbon et al., 1995; Wang et al., 2001), the LP (Kimura et al., 2012), the POm (Crabtree, 1996), and the dMGN (Crabtree, 1998, Kimura & Imbe, 2015) provide evidence of TRN projections to higher order thalamic nuclei. While most evidence comes from these anatomical studies, electrophysiological studies further demonstate TRN inhibitory projections to higher order thalamus. Intracellular recordings in POm neurons show IPSCs after photoactivation of caged glutamate in TRN (Lam & Sherman, 2007), indicating that these projections to higher-order thalamus are functional.

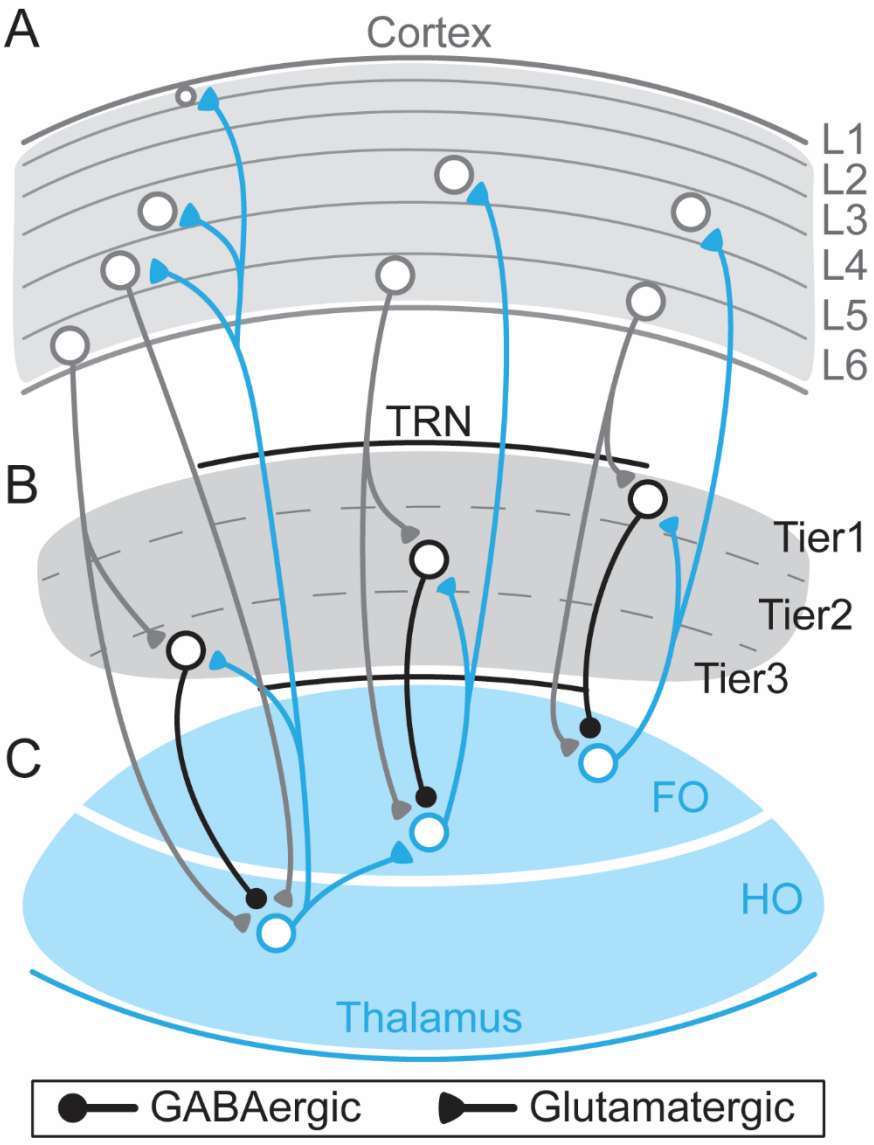
In addition to first and higher order thalamic nuclei, there are the various anterior and midline nuclei of the thalamus that are associated with the limbic system and receive inputs from the TRN. Expressing and activating the optogenetic protein channelrhodopsin (ChR2) in the rostroventral TRN resulted in the inhibition of spontaneous activity in anterior thalamic nuclei when recorded *in vivo* (Halassa et al., 2014). Other studies show that optogenetic activation of the rostrodorsal TRN inhibits spontaneous activity in midline thalamic nuclei in freely moving mice (Dong et al., 2019).

Taken together, the TRN generates inhibition in virtually all thalamic nuclei.

Inputs to TRN. The main sources of excitatory inputs onto TRN neurons are thalamocortical (TC) cells in the thalamus and corticothalamic (CT) cells in the cortex. TC cells in first order thalamic nuclei send sensory information through their afferents to layer IV of primary sensory cortex (Kharazia & Weinberg, 1994). These afferents pass through the TRN on their way to the cortex and branch off forming collaterals that target TRN cells (Pinault, 2004; Crabtree, 2018). Likewise, TC cells in higher order thalamic nuclei form collateral connections with TRN cells from their projections to cortical cells in layers I, IV, and V of primary and secondary sensory cortices (Mease, Metz, & Groh, 2016). In addition to the strong excitatory innervation from TC cells, the TRN also receives excitatory inputs from CT cells located in layer VI. In sensory cortices, layer VI neurons sends projections to both first order and higher order nuclei that form collaterals with TRN (Pinault, 2004; Crabtree, 2018). In contrast, anatomical evidence shows that CT projections from layer V only target higher order thalamic nuclei and do not form collaterals to TRN (Guillery, 1995; Crabtree, 2018). The majority of CT inputs to TRN come from primary and secondary sensory cortices (Crabtree, 2018), but recent evidence from optogenetic studies have found innervation from prefrontal cortex (Lee et al., 2019). Together, the connections between the cortex, the TRN, and the thalamus form the corticothalamocortical loop (CTC), with the TRN acting as the major regulator of CTC activity (Fig. 1.1).

The TRN also receives inhibitory inputs, although their sources are not well understood. Through the use of various structural labeling techniques and optogenetics, several types of GABAergic inputs have been identified, predominantly from subcortical areas. Using retrograde labeling in the TRN and staining for glutamic acid decarboxylase (GAD, a marker of GABAergic

Figure 1.1 The functional organization of the TRN in the cortico-thalamo-cortical loop. A. Cortex receives excitatory inputs from thalamocortical cells in both First Order (FO) and Higher Order (HO) nuclei. FO targets only layer IV (L4) whereas HO inputs target L1, L4, and L5. Layer VI cells send collaterals the TRN on their way to the thalamus **B.** Sensory TRN is organized into 3 laminal tiers, delineated by dashed lines as the borders are not defined structurally. Projections from Tier 1 and 2 cells only target FO nuclei whereas Tier 3 cells target both HO and FO (not shown). **C.** Thalamic cells in FO nuclei send collaterals to the upper tiers in the TRN on their way to the cortex. HO thalamic cells target other thalamic cells and their cortical projections form collaterals with TRN cells.


A
Cortex
L1
L2
L3
L4
L5
L6
TRN
B
Tier1
Tier2
Tier3
C
FO
HO
Thalamus
GABAergic
Glutamatergic

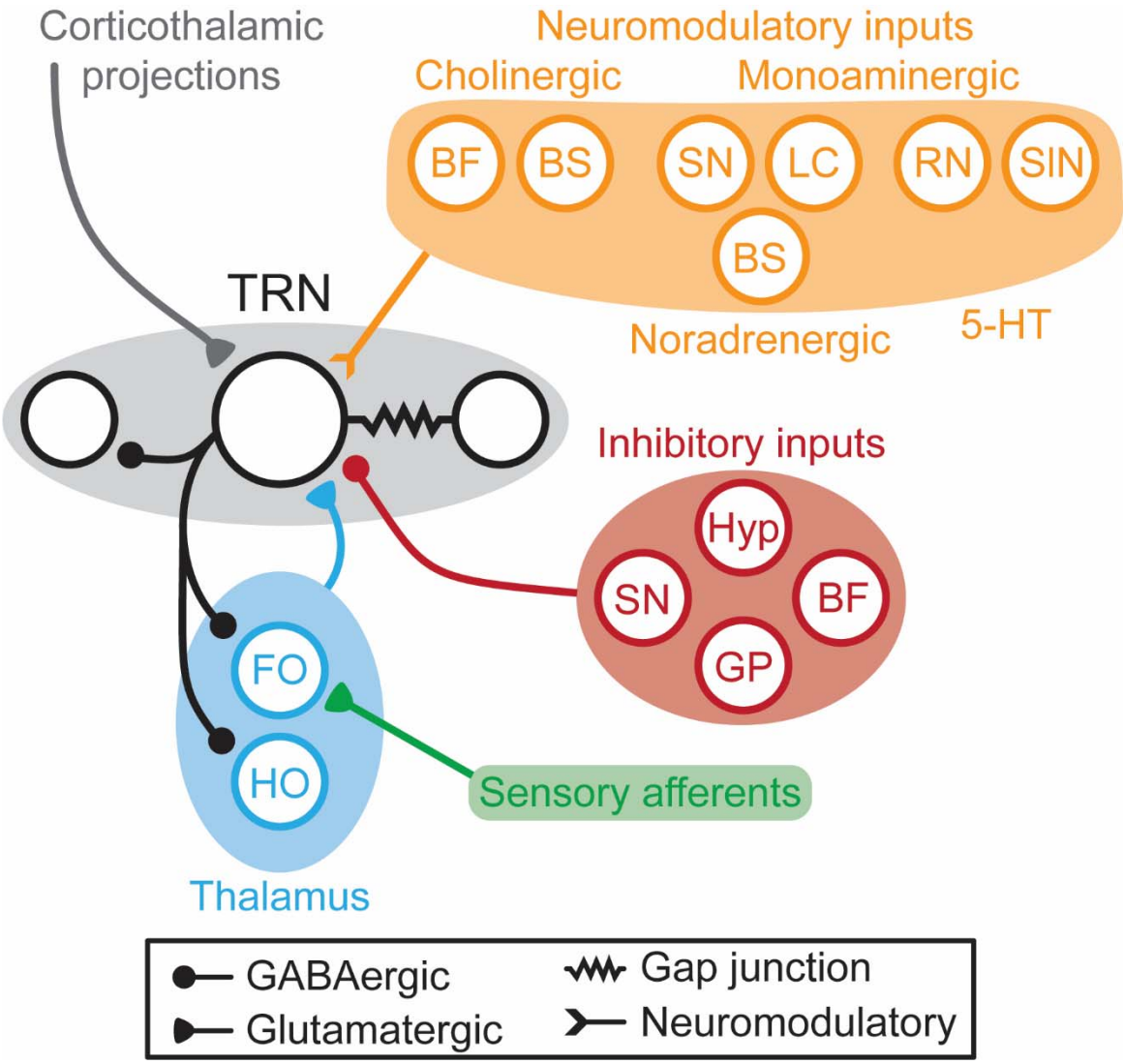
neurons), Bickford and colleagues (1994) identified GABAergic cells in the basal forebrain (BF) that project to the TRN. Anterograde labeling of GABAergic cells in the globus pallidus (GP) has shown inhibitory projections to the TRN (Asanuma, 1994). During intracellular recordings in anesthetized rats, stimulation of the GP resulted in inhibition of spontaneous TRN activity (Pazo et al., 2013) which further supports the notion that the GP sends inhibitory projections to the TRN. Optogenetic-assisted circuit mapping of the lateral hypothalamus revealed additional sources of inhibitory projections to the TRN. Following expression of ChR2 in the lateral hypothalamus, optical stimulation in the TRN revealed the generation of monosynaptic inhibitory current in TRN neurons but not thalamic neurons (Herrera et al., 2016). In addition to these areas, retrograde tracing from the TRN revealed labeling within the substantia nigra pars compacta (SNpc), and electrical stimulation of the SNpc resulted in inhibition of TRN activity, suggesting that the SNpc sends inhibitory inputs to the TRN (Paré et al., 1990). Together, these data suggest that the TRN receives inhibitory inputs project from several subcortical areas that shape TRN activity.

Neuromodulatory inputs to TRN. Neuromodulation shapes sensory processing and oscillatory activity by either enhancing the excitability or suppressing firing of TRN neurons (Mccormick, 1992). Neuromodulation of the TRN is largely mediated by cholinergic, monoaminergic, and peptidergic inputs, although there is evidence that other neurotransmitters may also play a role in the regulation of TRN activity. Immunohistochemistry (Parent & Descarries, 2008; Huerta-Ocampo et al., 2020) and optogenetic studies (Ni et al., 2016; Pita-Almenar et al., 2014b) have identified the basal forebrain, and the pedunculopontine tegmental and laterodorsal nuclei of the brainstem to be the main sources of cholinergic neuromodulation in the TRN. Additionally, retrograde tracing experiments identified noradrenergic inputs onto TRN cells that originate from the SNpC, locus coeruleus, and brainstem (Asanuma, 1992; Nagaeva & Akhmadeev, 2006; Hirata et al., 2006). While there is

evidence of dopaminergic signaling in the TRN, the source of these inputs has not been established (Huang et al., 1992). Serotonergic inputs identified from anatomical labeling originate within the raphe nucleus and the supralemniscal nucleus of the brainstem (Cropper et al., 1984; Rodríguez et al., 2011). Lastly, peptidergic signaling in the TRN comes from neuropeptide Y (NPY) that is released from neighboring TRN cells (Brill et al., 2007) in addition to cholecystokinin that is most likely released from corticothalamic and thalamocortical cells (Burgunder & Young III, 1990; Ingram et al., 1989; Schiffmann & Vanderhaegen, 1991; Cox et al., 1995). Taken together, the TRN receives distinct inputs from excitatory, inhibitory, and neuromodulatory sources, yet selectively innervates the thalamus (Fig. 1.2).

Intrinsic connections. Like GABAergic neurons in other brain areas (Connors & Long, 2004), TRN neurons are extensively interconnected by electrical synapses (Landisman et al., 2002; Landisman & Connors, 2005). Dual intracellular recordings of neighboring TRN cells show applying depolarizing currents to one cell results in depolarization of the other (Landisman et al., 2002). Furthermore, genetic knockouts indicated that the majority of electrical synapses are formed by gap junctions composed of connexin36 (Landisman et al., 2002; Lee et al., 2014). Dye coupling studies show that TRN neurons connected by gap junctions form distinct clusters (Lee et al., 2014). Lastly, in many areas of the brain the number of gap junctions decrease during development (Connors & Long, 2004). However, in the TRN gap junctions are more stable throughout development; evidence from immunohistochemistry studies have shown that TRN gap junctions are present from birth and remain throughout the animal's early stages of development (Parker et al., 2009). In addition to gap-junctions connecting TRN cells, there is conflicting evidence for intra-TRN chemical synapses.

Figure 1.2. Connectivity of TRN outputs and inputs onto TRN cells. The TRN (*black*) sends inhibitory projections to the majority of thalamic nuclei (*blue*) including both first order (FO) and higher order (HO) nuclei. In addition, there are intrareticular electrical and perhaps chemical connections. The thalamic nuclei and corticothalamic projections (*grey*) are the main source of excitatory inputs onto TRN. Inhibition onto the TRN comes from the lateral hypothalamus (Hyp), the substantia nigra pars compacta (SN), the basal forebrain (BF), and the globus pallidus (GP). Lastly, the TRN receives cholinergic neuromodulatory inputs from the BF and the pedunculopontine tegmental and lateral dorsal brain stem (BS) and monoaminergic neuromodulation from the SN, the locus coeruleus, dorsal ralphe nucleus (RN) and the supralemniscal nucleus of the brain stem (SlN).


Corticothalamic projections
Neuromodulatory inputs
Cholinergic
Monoaminergic
BF
BS
SN
LC
RN
SIN
BS
TRN
Noradrenergic
5-HT
Inhibitory inputs
Hyp
SN
BF
GP
FO
HO
Sensory afferents
Thalamus
GABAergic
Glutamatergic
Gap junction
Neuromodulatory

Using local glutamate uncaging to activate TRN neurons but not fibers of passage, bouts of IPSCs can be evoked in neighboring TRN neurons (Deleuze and Huguenard, 2004). However, studies using dual recordings suggest that while electrical synapses appear early in development (Hou et al., 2016), there appear to be absent during later stages (Landisman, 2002).

1.1.2. Functional organization of the TRN

The structural organization of the TRN shapes its regulatory control over different systems. This functional organization can be broken down into 5 major sectors: somatosensory, visual, auditory, motor, and limbic. Anatomical evidence shows that each distinct sectors of the TRN projects to a different functional system. First, the somatosensory sector of the TRN projects to the somatosensory thalamic nuclei, the VB (made up of the ventral posteromedial and ventral posterolateral nucleus), and the POm. The visual sector projects to the LGN, LP, and the pulvinar complex, which comprise the thalamic nuclei that process visual information (Kimura et al., 2012; Guido, 2018). The auditory portion of the TRN projects to the primary auditory thalamic nucleus, the ventral portion of the medial geniculate nucleus (MGN; Shosaku & Sumitomo, 1983), as well as the dorsal portion of the MGN (the higher order auditory nucleus) (Cruikshank et al., 2002; Lee & Sherman, 2008). The motor TRN projects to multiple thalamic nuclei, including the ventral anterior (VA), ventral lateral (VL) the central lateral (CL), medial dorsal (MD) nuclei (Lam & Sherman, 2015) and the anterodorsal nucleus (Crabtree, 2018). Lastly, the limbic TRN projects to the anterior thalamic nuclei (Halassa et al., 2014) and the paraventricular nucleus of the thalamus (PVT)(Dong et al., 2019; Lee et al., 2019).

While TRN neurons are thought to specifically target a single functional thalamic section, there is strong evidence that TRN allows for thalamic connectivity within or between distinct functional modalities. Activation of neurons in a given thalamic nucleus resulted in disynaptic

inhibition of other thalamic nuclei, either belonging to the same or a different functional modality (Crabtree et al., 1998; Crabtree & Isaac, 2002). Given that thalamic neurons lack synaptic connections within or across different thalamic nuclei, the TRN appears to be critical for the functional cross-talk between different thalamic modalities.

Each sensory sector is further subdivided in a laminar manner, which can determine whether the area will project to first order or higher order thalamic nuclei of the same sensory modality. There are three main laminar divisions within the TRN. Tier 1 is the most external division and forms the border with the internal capsule. Tier 2 is the middle portion of the TRN, bordered by Tier 1 and Tier 3. Finally, Tier 3 is the innermost layer of the TRN, located on the border of the thalamus. Anatomical (Pinault & Deschenes, 1998a; Pinault & Deschênes, 1998b), dye labeling (Lee et al., 2014) and immunohistochemical (Martinez-Garcia et al., 2020) evidence shows that cells within Tier 1 and Tier 2 of the TRN only project to the VB. In contrast, the cells in Tier 3 of the TRN serve as the main source of inhibition to the POm, but can also project to the VB.

1.1.3 State-dependent function of the TRN

Brain states are defined by the global pattern of activity in cortex measured using electroencephalogram (EEG) and imaging, allowing for the broad classification of behavioral states such as wakefulness and sleep (Kohn et al., 2009). Wakefulness is defined by high frequency desynchronized activity in EEG recordings. Behavioral and electrophysiological studies have found that high frequency activity exhibited in wakefulness is associated with sensory processing and reduces as an animal attends to a stimulus, and becomes more synchronous as the animal transitions to sleep. During the deep stages of sleep called slow-wave sleep (SWS) and under anesthesia, the activity of the TRN, thalamus and cortex become more synchronized and highly rhythmic (Amzica & Steriade, 1995; Beenhakker & Huguenard, 2009). The oscillatory pattern of activity exhibited

during SWS is thought to aid in memory consolidation and drive homeostatic maintenance (Marshall et al., 2006; Varga et al., 2016).

The function of the TRN changes depending on different brain states. During wakefulness and arousal, the TRN shapes sensory perception by regulating the sensory information passed between the thalamus and cortex. Computational modeling of TRN and thalamic activity during sensory processing established that the TRN acts as a high-pass filter for sensory information, which may help increase signal to noise, thereby enhancing attention (Scaglione & Moxon, 2006; Hartings et al., 2003). Lesion studies have shown that loss of TRN activity hinders performance on attention demanding tasks, further implicating the role of the TRN in attention (Bucherelli et al., 1993; Collery et al., 1993; Weese et al., 1999). Lastly, when TRN activity was inhibited either directly or disynaptically by optogenetic stimulation, performance declined on a multimodal sensory stimuli task that required selective attention to a single modality (Wimmer et al., 2015; Nakajima et al., 2019). In summary, the TRN aids in sensory processing and attention by filtering out low rates of activity from the thalamus, which in turn amplifies the relevant sensory information.

By contrast, during SWS, the TRN plays a crucial role in generating and maintaining slow oscillations that occur in the TRN, thalamus and cortex. Optogenetic and electrical activation of TRN neurons induces oscillatory activity and enhances memory formation following the act of learning a behavioral task, suggesting that the TRN plays a crucial role in both sleep oscillations and sleep dependent memory formation (Fuentealba et al., 2005; Lüthi, 2014; Latchoumane et al., 2017). Further evidence for a critical role of the TRN during sleep comes from animal models of Alzheimer's disease (AD). EEG recordings show increased incidence of awakening events and less cumulative time in SWS. By using ΔFosb (a molecular marker of activity) expression as a measurement of TRN activity Hazra and colleagues (Hazra et al., 2016) found decreased levels of ΔFosb in the TRN of a

mouse model of AD compared to wild-type mice. These data suggest that TRN hypofunction underlies sleep disruption and that TRN activation may promote the onset of SWS.

In summary, the TRN regulates sensory processing and generates oscillatory activity during sleep. While the various inputs shape the overall activity of TRN neurons, cell-intrinsic mechanisms also play a major role in shaping the TRN activity that enables the TRN to function in both arousal and sleep.

1.2 Structure and Function of TRN neurons

The firing properties and inputs that shape TRN neuronal activity and ultimately TRN function are in part determined by the spatiotemporal distribution of voltage and calcium-gated ion channels in TRN dendrites (Chklovskii, 2004). In this section I will describe the morphology of TRN neurons, the channels and conductances that shape TRN firing, how various fast and modulatory synaptic inputs alter TRN firing, and lastly, evidence for cell-intrinsic rhythmic activity.

1.2.1 Anatomy and molecular markers of TRN neurons

Various labeling and 3D reconstruction techniques highlight the unique organization and shape of the dendrites and cells bodies of TRN neurons (Pinault, 2004). Overall, findings from these studies suggest that extensive morphological heterogeneity among TRN neurons.

Somatodendritic morphology of TRN neurons. Previous studies have established three distinct dendritic morphologies among TRN neurons: one that is oriented along the dorso-ventral axis of the TRN, a second that extends parallel to the border between the TRN and thalamus, and a third that forms processes in all directions from the soma (Pinault & Deschenes, 1998; Spreafico et al., 1988). However, the existence of the latter morphological pattern is controversial as other groups (Lubke, 1993; Ohara & Havton, 1996) have shown that the dendrites of most TRN neurons are organized in

parallel bundles. These discrepancies are most likely due to examination of neurons in different parts of the TRN. Tightly grouped dendritic bundles have only been found in the dorsolateral TRN (Scheibel & Scheibel, 1972), suggesting that dendritic morphology is regionally distinct.

Early studies found TRN cell bodies to be predominantly large and elongated. More recent studies have identified three distinct shapes: large fusiform, small fusiform and small round (Spreafico et al., 1991; Spreafico et al., 1988), suggesting more extensive heterogeneity.

TRN cell types. Somatodendritic morphology indicates TRN heterogeneity but does not provide evidence for distinct cell types. Molecular markers are used extensively to define distinct subclasses of GABAergic cells throughout the brain. For example, studies on cortical interneurons identified at least 3 major classes of GABAergic cells (PV, SOM, 5-HT; Tremblay et al., 2016) each with several subclasses, based on molecular, morphological and electrophysiological features (Jiang et al., 2015). In the TRN, the large majority of cells express parvalbumin (PV), with a minority of neurons co-expressing markers such as calbindin (CB), somatostatin (SOM), cholecystokinin, (VIP), and neuropeptide Y (NPY) (Pinault, 2004; Clemente-Perez et al., 2017; Martinez-garcia et al., 2020). Recent work is starting to identify the location of cells in the TRN based on the molecular markers they express. PV expressing cells are found throughout the TRN (Csillik et al., 2005; Clemente-Perez et al., 2017; Martinez-Garcia et al., 2020). However, cells expressing other molecular markers show a more distinct distribution within the TRN. SOM expressing cells are typically found in the medial and lateral edges of the TRN whereas CB expressing cells are located in the center portions (Martinez-Garcia et al., 2020), suggesting that these two markers may help to distinguish cell types.

Taken together, TRN dendritic properties and the expression of several molecular markers indicate extensive neuronal diversity in the TRN, but additional efforts including single-cell RNA

sequencing (Jiang et al., 2015;Zeisel et al., 2015; Li et al., 2020) might be required to delineate distinct cell types.

1.2.2 *Basic firing properties of TRN neurons*

Like other thalamic neurons, TRN neurons show two distinct firing patterns determined by their resting membrane potential, (Kolaj et al., 2014; Weyand et al., 2001; Pinault, 2004) which has important functional consequences. *In vivo* recordings from rodents (Marks & Roffwarg, 1993) and cats (Steriade et al., 1986) show that TRN neurons can change the action potential firing from bursting to continuous action potentials (tonic mode). *In vitro* recordings have shown that this shift in firing mode is due to changes in the membrane potential (Llinas, 1988; Fuentealba & Steriade, 2005; Lee et al., 2007) and bursting typically occur when the membrane is hyperpolarized, while tonic firing is observed at more depolarized potentials. Electrophysiological recordings from TC neurons from *in vivo* and *in vitro* experiments similarly show both burst and tonic firing (Llinás & Jahnsen, 1982; Steriade et al., 1993a, 1993b; Llinás & Steriade, 2006; Sherman, 2001). Paired extracellular recordings show that bursts in TRN neurons are more robust, occurring more frequently and lasting longer with more spikes compared to TC burst (Pinault, 2003), suggesting that the underlying mechanisms may be different.

The major mechanism that mediates burst firing in the TRN is calcium currents generated by T-type calcium channels. T-type channels are low threshold channels, meaning that they are activated by small depolarizations from rest, but they also inactivate rapidly. Sequencing and DNA cloning have revealed 3 subtypes of T-type channels: Cav3.1, Cav3.2 and Cav3.3. T-type calcium currents in the TRN are mediated by Cav3.2 and Cav3.3, with the large majority of currents mediated by Cav3.3 (Astori et al., 2011). Calcium imaging of TRN dendrites showed strong calcium influx via T-type Ca channels in the distal dendrites while Ca influx in the soma and proximal dendrites were modest

(Crandall et al., 2010). The main function of T-type calcium channels is to increase the excitability of TRN neurons at hyperpolarized membrane potentials, resulting in a burst of action potentials following synaptic depolarizations.

TRN cells show distinct firing properties, further supporting the notion of heterogeneity within the TRN. Lee and colleges found three distinct firing properties in cells they recorded (Lee et al., 2007). About 38% of cells showed burst firing typical for TRN cells characterized by an "accelerando-decelerando" of action potentials. The second population of cells recorded (41%) expressed a smaller density of T-type current and showed non-burst firing, similar to the firing of SOM cells described recently (Clemente-Perez et al., 2017). Lastly, a smaller population of cells (21%) displayed "atypical bursting" characterized by an initially large amplitude spike and subsequently smaller amplitude but broader and longer-lasting spikes.

1.2.3 Several cell-intrinsic mechanisms shape TRN firing

While calcium currents through T-type calcium channels (I_T) have been well established as the main mechanism of TRN burst firing, several other currents modulate action potential activity.

Potassium channels. Potassium leak (K_{LEAK}) channels control the membrane potential in many neurons and in the TRN. K_{LEAK} channels shape excitability by changing the membrane potential and input resistance. Activation of G protein coupled receptors (GPCRs) leads to a block or closure of K_{LEAK} (Mathie, 2007) which depolarizes the resting membrane potential (Destexhe et al., 1994) and shifts the TRN cell into "tonic mode".

There are several calcium activated potassium channels in the TRN, with calcium-activated small conductance potassium (SK) channels being one of the main mechanisms for shaping burst firing in the TRN. In situ hybridization and immunolabelling have revealed that all four SK subunits are expressed in the mammalian brain (Stocker & Pedarzani, 2000; Turner et al., 2014), with SK2

being the major SK channel expressed throughout TRN cells (Wimmer et al., 2012). The function of SK channels in shaping the hyperpolarization is two-fold. First, its activation will terminate the burst firing. Second, SK-mediated hyperpolarization to membrane potentials well below -60 mV allows for the de-inactivation of T-type calcium channels. In some systems, T-type calcium and SK channels form so-called micro- or nano domains (Fakler & Adelman, 2008; Turner & Zamponi, 2014) but their presence has not been conclusively established for TRN neurons (Cueni et al Nature Neuro).

Lastly, voltage-gated potassium channels play a role in shaping TRN firing. Like most PV expressing cells throughout the brain, TRN neurons highly express voltage gated potassium channels of the Kv3 subfamily (Weiser et al., 1994; 1995; Chang et al., 2007). Genetic ablation of Kv3.1 and Kv3.3 resulted in a decrease of action potentials in bursts during intracellular recordings in thalamic slices and in extracellular recordings in freely behaving mice (Espinosa et al., 2008). This indicates that voltage dependent potassium channels are important for shaping bursts and spike fidelity by increasing the afterhyperpolarization.

R-type calcium channels. While T-type calcium channels are necessary for burst firing in the TRN, R-type calcium channels may be critical for T-type channel activation. Pharmacological antagonism and genetic knockout of R-type calcium channels prevented burst firing, suggesting that the depolarization from R-type channels results in T-type calcium channel activation (Zaman et al., 2011).

HCN channels. One major channel that shapes TRN firing is the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. HCN channels are typically activated at membrane potentials well below the resting membrane potential and are closed at more depolarized potentials (Wahl-Schott & Biel, 2009). Injecting a hyperpolarizing current in TRN cells results in a slight membrane

depolarization due to currents through HCN channels (Mccormick & Pape, 1990). Therefore, HCN channel activation can trigger activation of other low threshold channels like T-type.

Persistent sodium current (I_{NAP}). In addition to generating tonic and burst firing, TRN neurons can generate afterdepolarizations following spiking, which in turn can lead to sustained depolarizations termed plateau potentials. The mechanisms underlying ADPs and plateau potentials remain poorly understood. While the large majority of voltage-gated Na currents underlying fast action potentials are fast and transient, a small component shows little inactivation, generating a persistent Na current (I_{NAP}) which might underlie plateau potentials. In a fraction of TRN cells intracellular recordings revealed long-lasting plateau potentials. Adding blockers of voltage-dependent sodium channels to the bath or internal solution led to a block suggesting that the plateau potential is mediated by I_{NAP}. (Kim & Mccormick, 1998; Fuentealba et al., 2005).

Calcium-activated non-selective cation current (I_{CAN}). A second proposed mechanism underlying plateau potentials are long-lasting calcium activated non-selective cation currents (I_{CAN}). I_{CAN} has been described for neurons in cortex (Zylberberg & Strowbridge, 2017; Lei et al., 2014) hippocampus (Mrejeru et al., 2011), brainstem (Ben-Mabrouk & Tryba, 2010; Picardo et al., 2019) and several other areas. The molecular mechanisms underlying I_{CAN} remain controversial (Zylberberg & Strowbridge, 2017), see *1.2.5.*

1.2.4 Synaptic and state-dependent control of TRN firing

Unlike other neurons, TRN cells do not show spontaneous activity. Therefore, TRN neuronal firing is entirely driven by synaptic inputs. The different inputs onto TRN cells described in section 1.1 can alter the activity based on the types of currents they generate, and where the inputs terminate on TRN cells. Additionally, during the different brain states of sleep and wakefulness the TRN has different firing patterns regulated by various neuromodulatory and cell intrinsic properties. So

understanding how the various inputs shape firing and state dependent activity will offer insights into how different inputs can shape TRN function.

As discussed in section 1.1.1, thalamocortical projections from both first order thalamic relay and higher order thalamic cells send collateral projections to TRN neurons. Intracellular recordings of thalamic relay and TRN pairs showed that thalamic inputs typically generate very large AMPA-mediated excitatory postsynaptic currents (EPSCs) (Gentet & Ulrich, 2003). Furthermore, stimulation of thalamic relay cells resulted in burst of action potentials in the TRN, demonstrating that single thalamic neurons can reliably drive activity in postsynaptic TRN cells. Similarly, selective activation of higher order thalamic cells using caged-glutamate and photostimulation resulted in robust EPSCs in TRN neurons (Lam & Sherman, 2011). Of note, structural labeling revealed thalamic inputs mainly project onto the proximal dendrites of TRN cells, forming large synapses that targeted only a few TRN cells (Liu & Jones, 1999), suggesting the thalamus has a selective drive of the TRN.

Activation of cortical inputs evokes AMPA- and NMDA- mediated responses (Crandall et al., 2015) that are larger in TRN as compared to neighboring relay nuclei (Golshani et al., 2001) Optogenetic activation of corticothalamic fibers can generate large facilitating glutamatergic responses in TRN neurons (Jurgens et al., 2012; Crandall et al., 2015). In tonic mode, such facilitating synaptic input lead to sustained TRN activation, while in burst mode, firing is decreased over time, presumably due to T-type inactivation and a resulting decrease in burst firing (Crandall et al., 2015). In addition, to generating fast postsynaptic responses, high-frequency corticothalamic stimulation using extracellular electrodes can lead to the recruitment metabotropic glutamate receptors mGluRs (Alexander & Godwin, 2006; Long et al., 2004) though this remains to be confirmed using optogenetic techniques. Pharmacological studies using agonist applications have identified Group I mGluRs depolarizing TRN neurons while in a smaller fractions of neurons hyperpolarizations can

occur due to the activation of Group II mGluRs. Selectively blocking Group I mGluRs in the presence of a nonselective mGluR agonist depolarized TRN cells by blocking K_{LEAK} channels whereas selectively blocking Group II mGlurs hyperpolarize the cell by opening a potassium channel (Cox & Sherman, 1999). Contrary to thalamic-reticular inputs, the majority of cortical inputs target the distal dendrites of TRN neurons, forming smaller synapses and typically targets multiple TRN cells (Liu & Jones, 1999).

Action potential activity in the TRN is modulated by cholinergic inputs. Initially thought to modulate TRN activity by volume transmission, immunolabeling of cholinergic afferents revealed varicosities forming on TRN dendrites, and electron microscopy showed cholinergic inputs form synapses with TRN neurons (Parent & Descarries, 2008). Several studies combining pharmacological or immunohistochemical techniques with electrophysiology have implicated that α7 (Ni et al., 2016) and α4β2 (Sun et al., 2013) nicotinic acetylcholine receptors (nAChR), and M2 muscarinic acetylcholine receptors (mAChR) are expressed in TRN neurons (Sun et al., 2013; Pita-Almenar et al., 2014b). Activation of cholinergic inputs from the basal forebrain and brainstem evoke a biphasic response with a fast excitatory current followed by a slow inhibitory current mediated by α4 β2 nAChR and M2 mAChR respectively. While nAChRs are ionotropic, mAChRs are metabotropic and lead to the activation of G-protein coupled inward rectifying potassium (GIRK) channels causing a slow inhibitory response. The strength of cholinergic inputs can be altered by postsynaptic mGluR activation (Sun et al., 2016) suggesting heterosynaptic interactions between glutamatergic and cholinergic afferent activity to TRN.

Brain states influence the activity patterns of TRN neurons. Extracellular recordings from freely moving animals during sleep-wake transitions revealed that TRN neurons typically fire in burst mode while the animal is asleep and switch to tonic mode when the animal is in the awake state

(Steriade et al., 1986; Steriade et al., 1993c). However, burst firing can be recorded in awake animals during sensory processing of visual stimuli (Reinagel et al., 1999; Swadlow & Gusev, 2001) indicating that burst firing is not exclusive to sleep. The change in the resting membrane potential underlying changes in firing mode is due to the shift in activity of several neuromodulatory inputs during brain state transitions (Diekelmann & Born, 2010; Lee & Dan, 2012; Adamantidis et al., 2019). Increases in monoamines and acetylcholine during wakefulness block K_{LEAK} channels through GPCRs and TRN recordings in awake animals show TRN cells typically fire tonically (Steriade et al., 1993c). Pharmacological manipulations of neuromodulation revealed the state-dependent changes in activity are caused by changes in the resting membrane potential by closing of K_{LEAK} channels (Mccormick, 1992; Alexander & Godwin, 2006). In contrast to wakefulness, the open state of K_{LEAK} and a hyperpolarized membrane potential is typical of TRN neurons during sleep. Evidence from *in vivo* recordings in sleeping and anesthetized animals shows that the hyperpolarized membrane potentials results in rhythmic bursts action potentials which are thought to be maintained in a cell intrinsic manner (Marks & Roffwarg, 1993). In summary, TRN activity is shaped by a diverse set of inputs that drive burst and tonic activity in a state dependent manner.

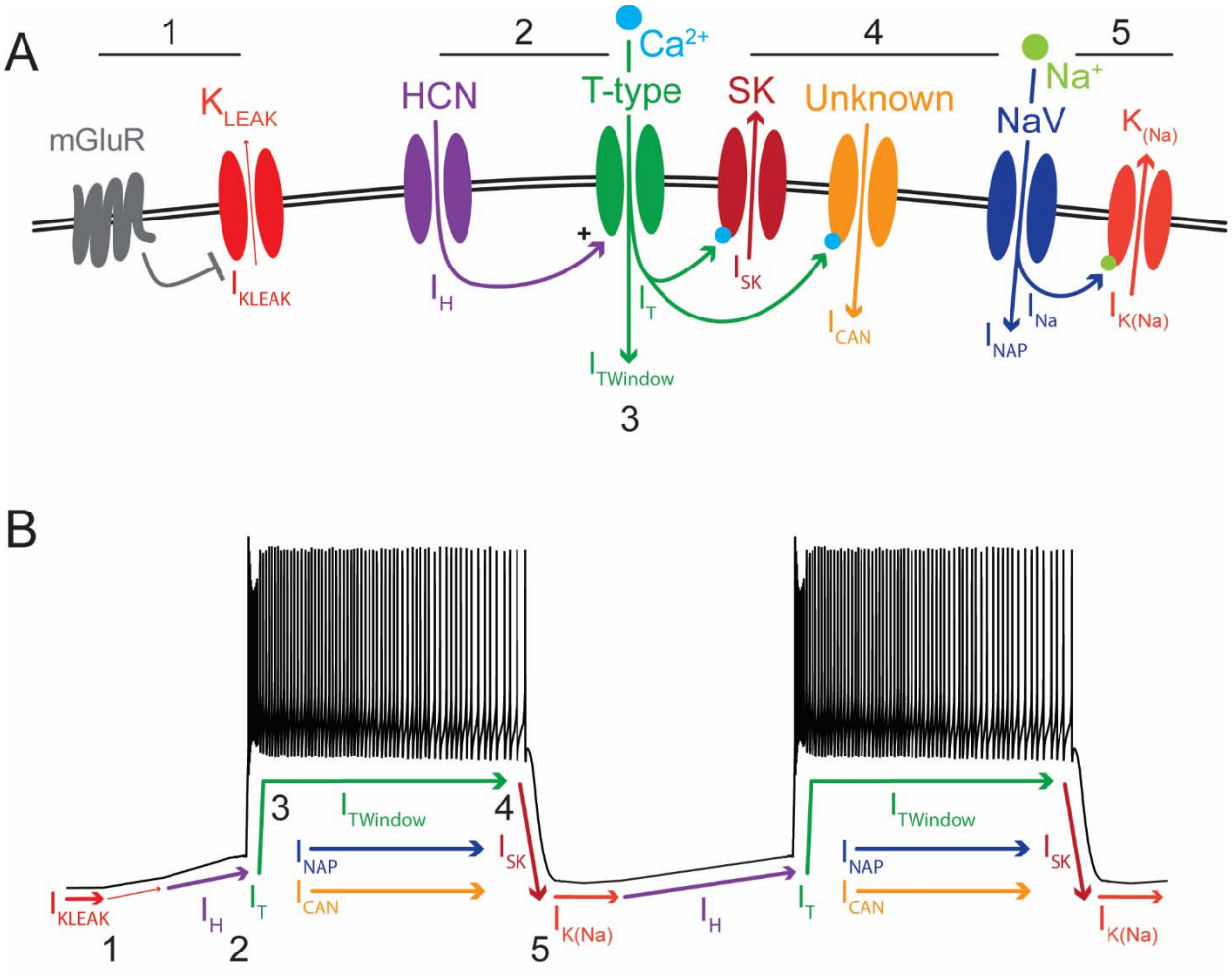
1.2.5 Cell-intrinsic rhythms in the TRN

Thalamus including TRN shows different types of oscillatory activity during sleep. Thalamic oscillations observed are sleep spindles (10-14 Hz), delta oscillations (1-4 Hz), and slow oscillations (<1Hz). Both spindles and delta oscillations can be nested within slow oscillations (Crunelli & Hughes, 2010; Crunelli et al., 2018), implicating a crucial role of slow oscillations. Intracellular recordings from anesthetized animals found these slow oscillations in the TRN and TC neurons (Nuñez et al., 1992; Steriade et al., 1993; Crunelli & Hughes, 2010; Crunelli et al., 2012). Under these conditions, oscillations in the TRN and TC shift between periods of sustained depolarizations with

persistent firing of action potentials and an underlying plateau potential (UP states), and periods of prolonged hyperpolarization and quiescence (DOWN state). Isolated TRN and thalamic *in vitro* slice preparations have also revealed this slow oscillatory activity, suggesting that the TRN and TC can generate oscillatory activity in a cell-intrinsic manner (Hughes et al., 2002; Blethyn et al., 2006).

Seminal work by Crunelli and colleagues (Hughes et al., 2002; Blethyn et al., 2006) has isolated several of the cell-intrinsic mechanisms that generate and shape slow oscillations in single TRN and TC cells, triggered by the application of Group I mGluR agonists. As stated previously, activation of mGluRs results in a G-protein mediated reduction of K_{LEAK} conductances. Under these conditions, with a reduced K_{Leak}, slow oscillatory activity is generated through the interplay of T-type calcium channels, HCN channels, potassium channels, and an unknown channel that generates an I_{CAN} (Hughes et al., 2002; Blethyn et al., 2006; Nilius, et al., 2007; Bal & Mccormick, 1993; Fig. 1.3). These findings suggest that T-type channels in both TRN and TC neurons generate a window current (T_{Window}) resulting prolonged depolarizing plateau potential (Hughes et al., 1999). T_{Window} currents occur because the membrane potential is depolarized enough for some current to be activated, but not sufficiently depolarized to achieve complete inactivation at steady-state. In this state, T_{Window} results in plateau potentials lasting up to several seconds and in TRN neurons leads to persistent action potential firing for the duration of the plateau. While an I_{CAN} has been implicated in plateau potentials in the thalamus and TRN, the exact role of these currents is not well understood. Evidence from modeling suggests that I_{CAN} shapes the plateau potential by facilitating the membrane depolarization during the plateau potential, but by itself is not necessary for the generation of the plateau potential (Hughes et al., 2002; Blethyn et al., 2006). In TC cells, the K_{LEAK} is the only mechanism known to hyperpolarize the cell during these oscillations; however, in the TRN, pharmacological antagonism and modeling have shown that SK and sodium-activated potassium

Figure 1.3. mGlur-mediated cell-intrinsic mechanisms of TRN oscillations. **A.** shows the major mechanisms that have been implicated in generating cell-intrinsic oscillations. **B.** shows how each conductance generated by the mechanisms shown in A are involved in oscillatory activity. In states where K_{LEAK} (*red*) is reduced by mGluR (*grey*) activation (1), the cell becomes slightly depolarized, activating HCN (*purple*). Depolarization from HCN activates T-type channels (*green*)(2) and generates a T_{Window} current that results in a plateau potential (3). Additionally, as the cell is depolarized NaV channels (blue) are activated as is an unknown channel that generates an I_{CAN} (*yellow*) that are thought to facilitate the plateau. The calcium from T-type channels activates SK channels (*dark red*)(4) and NaV activate K_{Na} (*orange*)(5) hyperpolarizing the cell, shaping the duration of the AHP, and allowing for HCN to be activated, restarting the oscillation. Data in B are mock data based on my recordings.


A
1
2
3
4
5
mGluR
K LEAK
I KLEAK
HCN
I H
Ca2+
T-type
I T
I TWindow
SK
I SK
Unknown
I CAN
Na+
NaV
I Na
I NAP
K (Na)
I K(Na)
B
I KLEAK
I H
I T
I TWindow
I NAP
I CAN
I SK
I K(Na)

(K_{Na}) channels also shape the hyperpolarizing phase (Hughes et al., 2002; Blethyn et al., 2006; Crunelli et al., 2012). The hyperpolarization by potassium channels allows for the activation of HCN channels, and antagonism of HCN channels resulted in loss of the cell-intrinsic rhythms suggesting HCN channels are necessary for the generation of rhythmic activity. This is likely due to the fact that depolarization from HCN channels results in activation of T-type calcium channels, initiating another UP State and continuing the oscillation (Mccormick & Pape, 1990; Bal & Mccormick, 1993).

While Crunelli and colleagues were able to isolate some of the mechanisms of TRN slow oscillations, others mechanism such as those that generates the I_{CAN} are unknown. Therefore, the exact role I_{CAN} has on shaping these oscillations cannot be determined. Given that the only evidence for I_{CAN} in shaping these plateau potentials during oscillations comes from modeling of cellular rhythms, it is likely that the role of I_{CAN} is understudied. Furthermore, these oscillations occur under non-physiological conditions i.e. in the presence of mGluR agonists. Lastly, in these studies the synaptic inputs from thalamus were pharmacologically blocked. Given that the TRN receives powerful thalamoreticular inputs, it is important to investigate their potential role in generating slow oscillations.

1.3 Structure and Function of TRN microcircuits

The TRN is interconnected with the thalamus and cortex forming the corticothalamic loop. While both inputs drive TRN activity, shaping sensory processing and oscillatory activity, the TRN sends projections only to thalamus creating an intrathalamic network of reciprocal connections. These short-range connections between TRN cells and between the TRN and thalamus can be further broken down into microcircuits (Pinault, 2004). Only consisting of a small handful of cells, these microcircuits are functionally important as powerful generators of oscillatory activity and gates of sensory processing of the TRN. In this section, I will describe the two microcircuits of the TRN, intra-

reticular and intra-thalamic, and describe their structure and function during sensory processing and how they generate oscillatory activity in the TRN.

1.3.1 Intra-reticular functional organization

The intra-reticular connections are important for shaping output of TRN cells, there by shaping the activity and function TRN microcircuits. As discussed previously these connections are made up of electrical synapses formed by gap junctions and chemical inhibitory synapses.

Gap junctions allow for the flow of ions and small molecules between localized cells and can synchronize the activity of coupled cells (Hormuzdi et al., 2001; Söhl, et al., 2005). In the TRN, several studies have shown that gap junctions between local TRN neurons can coordinate both fast action potential activity (Landisman et al., 2002) and slower subthreshold membrane oscillations (Long et al., 2004). Importantly, coupling mediated by gap junctions can be altered through long-term plasticity (Landisman & Connors, 2005; Coulon & Landisman, 2017). Exogenous activation of mGluRs results in a long-term depression of currents carried by gap junctions resulting in a loss of coordination of spiking between dual recorded cells (Landisman & Connors, 2005). Additionally, plasticity of gap junctions can also occur in an activity dependent manner. Dual intracellular recordings show that after burst firing was evoked in one cell, both electrically coupled cells had a reduced calcium current, indicative of long-term depression (Haas et al., 2011). Computational modeling further supports that both long-term depression and long-term potentiation of electrical coupling is activity-dependent (Pernelle et al., 2017).Sparse firing lead to long-term potentiation of gap junctions which resulted in increased occurrences of spindle oscillations. Taken together, gap junctional coupling is highly plastic and works to synchronize the activity TRN cells, resulting in coordinated inhibition of the thalamus.

TRN neurons form small clusters that are connected by gap junctions (Landisman & Connors, 2005; Landisman et al., 2002). The laminar location and the organization of these clusters within the TRN can determine their function. Dye coupling revealed that the majority of these clusters are elongated and span within a single tier (Lee et al., 2014). Additionally, broad clusters were labeled that spanned across multiple tiers in the TRN. Consistent with findings by Pinault, axonal labeling of the clusters showed the laminar location determined the projections of the cells within the cluster. Clusters that spanned the outer most and the central shells projected to first order thalamus whereas clusters in the inner most shell had axonal projections terminating in higher order thalamus. Interestingly, the clusters that spanned multiple tiers in the TRN had projections to both first order and second order nuclei. Taken together, these data implicate that the elongated clusters coordinate selective inhibition of distinct thalamic nuclei. In contrast, the broad clusters may act to coordinate widespread inhibition of the thalamus, which may target non-important sensory relay cells enhancing sensory processing or enhance the synchrony of oscillations in TRN microcircuits.

As mentioned previously, there is evidence of intra-TRN chemical synapses (Deleuze and Huguenard, 2006). There functional role is thought to lie in a reduction of synchronous activity. This is supported by increased synchrony in extracellular recordings of TRN from thalamic slices following genetic ablation of TRN $GABA_A$ receptors (Huntsman et al., 1999; Sohal, et al., 2000). Taken together the intrareticular connectivity can modulate TRN cells via GABAergic synapses and enhances the synchrony of oscillatory activity through gap junctions. While modeling studies have suggested that TRN can generate self-sustained rhythmic oscillations (Bazhenov et al., 1999), it is the connectivity between the TRN and thalamus that plays a large role in generating distinct types of oscillations in the thalamus.

1.3.2 Intrathalamic microcircuitry of the TRN and thalamus

The TRN sends projections to the thalamus that in turn send reciprocal connections back to the TRN forming thalamic microcircuits. These circuits provide a source of feedback inhibition to the thalamus and act as generator of network activity that is important for sensory processing and rhythmic network activity. Evidence for the functional importance of intrathalamic microcircuits during sensory processing has been obtained for several modalities intrathalamic microcircuits have been identified based on their molecular markers (Martinez-Garcia et al., 2020; Li et al., 2020). In situ hybridization revealed two non-overlapping populations of TRN cells based on expression of CB and SOM (Martinez-Garcia et al., 2020). Cells expressing CB were found to only be in center of the TRN and projected only VB thalamus, whereas SOM expressing cells were located on the edges of the TRN and selectively targeted POm. Furthermore, intracellular recordings of these two TRN cells showed the excitatory inputs from these thalamic nuclei were distinct. VB inputs onto CB-TRN cells had fast excitatory currents with strong short-term depressing. In contrast, POm onto SOM-TRN cells had slower, facilitating excitatory currents, implicating that these microcircuits can be dynamic. In that regard, *in vivo* recordings from the anterior and dorsal portions of the TRN revealed two state-dependent networks (Halassa et al., 2014). One network participated in sleep rhythms, where the other showed increased activity during arousal. Taken together, the data indicate that these microcircuits are important for state dependent regulation of the thalamus.

One major aspect of these reciprocal connections between TRN and thalamic is that they form a closed loop (Pinault, 2004). That is, the sensory relay thalamic neurons send projections to TRN neurons which in turn sends inhibitory projections back to the same thalamic cells. While there are reciprocal connections between the TRN and thalamus the number of connections differs. The TRN sends wide projections into the thalamus diverging onto multiple thalamic cells. For a given thalamic neuron, multiple TRN neurons form convergent input. The thalamic inputs onto TRN cells are far

sparser, resulting in an asymmetry of connectivity between the TRN and thalamus (Pita-Almenar et al., 2014b). It is important to point out here that this asymmetry between the TRN to TC and TC to TRN connections can lead to open loops between the thalamus and TRN, suggesting that the TRN has a larger regulatory role over the thalamus.

1.3.3 Intrathalamic microcircuits control oscillatory activity of the TRN

The reciprocal connections between the TRN and thalamus are important for maintaining oscillatory activity of the TRN, particularly found during distinct phases of SWS. GABAergic projections from the TRN result in rebound bursts in relay neurons that in turn drives TRN neurons resulting in network driven oscillations between the TRN and thalamus. Both ionotropic $GABA_A$ receptors and $GABA_B$ receptors in thalamic neurons play a role in the generation of this oscillatory activity (Kim et al., 1997). Pharmacological antagonism of $GABA_B$ reduced oscillatory activity recorded in intracellular recordings from thalamic slices (Huguenard & Prince, 1994), suggesting that $GABA_B$ receptors contribute to the slow network oscillations. Given that there are distinct oscillations that occur in the thalamus, it is important to review what is known about the spindle and slow (< 1Hz) oscillations and the role of thalamic microcircuits.

Spindle oscillations. In the absence of TRN or thalamic inputs, spindle activity is lost (Steriade et al., 1985), suggesting that the connections between the TRN and thalamus play an important role in spindle oscillations. Activating the TRN resulted in sleep spindle activity, suggesting that one of the functions of the TRN during sleep is to generate sleep spindles (Kim et al., 2012; Halassa et al., 2011). However, not all cells in the TRN are involved in generating spindle-like activity. *In vivo* recordings show that a small population of cells with bistability or a plateau potential (~33%) may be the generators of sleep spindles (Fuentealba et al., 2005). Given the asymmetry of connectivity between the TRN and thalamus, it is likely that the few cells shown to generate spindles activate a

larger population of thalamic cells thereby recruiting more TRN cells. These studies suggest that a small population of cells within the TRN act as generators of spindle activity and the reciprocal connections between the TRN and thalamus maintain spindle activity.

Slow oscillations. The TRN along with the cortex and thalamus show slow oscillatory activity in sleep (Crunelli & Hughes, 2010). The slow (<1 Hz) oscillations, are characterized by the UP and DOWN states from EEG and intracellular recordings in TRN (Steriade et al., 1993a), Thalamus (Steriade et al., 1993b), and cortex (Steriade & Amzica, 1996; Steriade et al., 2001) during SWS and under anesthesia. The mechanisms for generating slow oscillations have been examined in detail in the cortex, but less so in the TRN and thalamus. In cortex, decortications studies have established that the reciprocal networks in the cortex generate and maintain the UP and DOWN states, and that layer V cortical cells may be the initiator of these oscillations (Steriade & Amzica, 1996; Sanchez-Vives & McCormick, 2000; Lőrincz et al., 2015). However, as outlined above, the slow oscillations in the TRN are thought to be maintained in a cell-intrinsic manner, and initiated by mGluR activation mediated by sustained activation of corticothalamic inputs. Nevertheless, recent work has emerged showing that in the absence of cortical inputs the thalamus can generate slow oscillatory activity during sleep states (Adamantidis et al., 2019), suggesting that such activity patterns can be generated by mechanisms intrinsic to the thalamus.

Given the interconnectivity of the TRN microcircuits with the thalamus and the fact that these circuits are capable in generating oscillatory activity, it stands to reason that these connections play a significant role in the slow oscillatory activity observed in the TRN. Yet, no study to date has examined the role of the intrathalamic network in driving slow oscillations. Therefore, it is not quite clear how the interplay of the cell-intrinsic and network properties work together to generate and mediate these oscillations as the underlying mechanisms are poorly understood.

To that end I set forth with two aims for this dissertation:

(1) Determine the role of the intrathalamic microcircuit in generating slow oscillations in the thalamus.

(2) Identify the mechanisms that shape TRN neuronal firing.

I addressed these aims by performing recordings in horizontal slices of somatosensory thalamus of adult mice. Surprisingly, I observed highly robust slow oscillatory activity, which was driven by fast synaptic transmission but did not require mGluR activation. Furthermore, I found that a significant number of TRN neurons displayed synaptically evoked persistent firing (PF), which could also be evoked by brief depolarizing current steps in the absence of synaptic signaling. PF was triggered by calcium influx through T-type calcium channels and generated by long-lasting plateau potentials mediated by TRPM4 conductances. My findings highlight how intrinsic properties of TRN neurons and intrathalamic synaptic circuits interact to generate slow thalamic oscillatory activity.

Chapter 2: Materials and Methods

Reproduced with permission from O'Malley, J.J., Seibt, F., Chin, J., & Beierlein, M. (2020) TRPM4 conductances in thalamic reticular nucleus neurons generate persistent firing during slow oscillations. *J. Neurosci.* 40(25), doi: 10.1523/jneurosci.0324-20.2020. (O'Malley, Seibt, Chin, & Beierlein, 2020)

2.1 Animals

I employed C57BL6/J mice (JAX Laboratories, Stock No: 000664) unless specified otherwise. For some experiments, I used TRPC1,4,5,6 general quadruple knockout mice (Sachdeva et al., 2018) kindly provided by Dr. Michael Zhu (McGovern Medical School at UTHealth) or TRPC3 general knockout mice (Hartmann et al., 2008) kindly provided by Dr. Oleh Pochynyuk (McGovern Medical School at UTHealth). To optogenetically activate cholinergic inputs to TRN, I used bacterial artificial chromosome (BAC)-transgenic mice expressing channelrhodopsin (ChR2) under the control of the choline acetyltransferase (ChAT) promoter (ChAT–ChR2–EYFP; JAX Laboratories, Stock No: 014546, Zhao et al., 2011). All animals used in this study were treated following procedures in accordance with National Institutes of Health guidelines and approved by the University of Texas Health Science Center at Houston (UTHealth) animal welfare committee.

2.2 Slice Preparation

Brain slices derived from adult animals (P90-120) were prepared as described previously (Ting et al., 2014). Briefly, mice of either sex were anesthetized using isoflurane and perfused using an ice cold N-Methyl-D-Glutamine (NMDG) based solution saturated with 95% O_2–5% CO_2 consisting of the following (in mM): 92 NMDG, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 30 $NaHCO_3$, 20 glucose, 20 HEPES, 2 thiouera, 5 Na-ascorbate, and 3 Na-pyruvate. Brains were

removed and horizontal slices (300 μm) were cut in ice-cold NMDG-based solution using a VT1200 S vibratome (Leica). Slices were then held in NMDG-based solution maintained at 35°C for ~12 minutes before being transferred to a modified artificial cerebrospinal fluid (ACSF) held at room temperature, consisting of the following (in mM): 92 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 30 $NaHCO_3$, 25 glucose, 20 HEPES, 2 thiouera, 5 Na-ascorbate, and 3 Na-pyruvate. Slices from younger animals (P13- 38) were prepared as described previously (Agmon & Connors, 1991). Briefly, mice were anesthetized using isoflurane and then decapitated. Slices were cut in ice-cold cutting solution saturated with 95% O_2-5% CO_2 consisting of the following (in mM): 213.4 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$, and 11 glucose. Slices were then transferred to ACSF consisting of the following (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose, incubated at 35°C for 20 minutes and then held at room temperature until used for experiments.

2.3 Electrophysiological Recordings

Recordings were performed in a chamber perfused with ACSF held at 32-34°C using a Warner Instruments TC-324B in-line heater. Cells were visualized via infrared differential interference contrast (IR-DIC) under an Olympus BX51WI 120 microscope equipped with a Dage-MTI IR-1000 camera. Whole-cell recordings were obtained using glass pipettes with a tip resistance of 3-5 MΩ. For current clamp recordings in TRN, I used a potassium-based internal solution consisting of (in mM): 133 K-Gluconate, 1 KCl, 2 $MgCl_2$, 0.16 $CaCl_2$, 10 HEPES, 0.5 EGTA, 2 Mg-ATP, and 0.4 Na-GTP (adjusted to 290 mOsm and pH 7.3). For voltage clamp recordings of inhibitory postsynaptic currents in neurons of the ventrobasal nucleus of the thalamus (VB), recording pipettes were filled with a cesium-based internal solution consisting of (in mM): 120 $CsMeSO_3$, 1 $MgCl_2$, 1 $CaCl_2$, 10 CsCl, 10 HEPES, 3 QX-314, 11 EGTA, 2 Mg-ATP, and 0.3 Na-GTP (adjusted to 295 mOsm and pH

7.3). For loose patch recordings of TRN neuronal firing, glass pipettes (3-5 MΩ) were filled with ACSF, and recordings were obtained in voltage-clamp mode, at seal resistances of 50–200 MΩ. To minimize any influence on the cell membrane potential, the holding voltage was adjusted continually to maintain a holding current near 0 pA (Perkins, 2006). In experiments probing TRN oscillatory activity, glutamine (0.3 mM) was added to the ACSF to prevent a rundown of network activity (Bryant et al., 2009; Pita-Almenar et al., 2014b). For experiments examining the mechanisms of PF, the bath contained NBQX. In some experiments I replaced extracellular NaCl with equimolar NMDG and used an internal solution consisting of (in mM): 133 K-Gluconate, 1 KCl, 4 NaCl, 2 $MgCl_2$, 0.16 $CaCl_2$, 10 HEPES, 0.5 EGTA, 2 Mg-ATP, and 0.4 Na-GTP (adjusted to 290 mOsm and pH 7.3). For optogenetic activation of cholinergic afferents I used 1 ms pulses of blue light generated by an LED light source (UHP-T-450-EP, Prizmatix) delivered through a 60x, 0.9 NA water-immersion objective (Olympus) and centered locally in the TRN.

NBQX, apamin, nimodipine, TTX, 2-Aminoethoxydiphenylborane (2-APB), flufenamic acid (FFA), 9-phenthranol, glibenclamide, 4-Chloro-2-[[2-(2-chlorophenoxy)acetyl]amino]benzoic acid (CBA), JNJ 16259685, MTEP hydrochloride, and atropine were obtained from R&D Systems. ω-conotoxin MVII and TTA-P2 were obtained from Alomone labs. All other chemicals were obtained from Sigma-Aldrich.

2.4 Data acquisition

Recordings were made using a Multiclamp 700B amplifier (Molecular Devices), filtered at 3–10 kHz, and digitized at 20 kHz with a 16-bit analog-to-digital converter (Digidata 1440A; Molecular Devices). Data were acquired using Clampex 10.3 software (Molecular Devices) and analyzed using custom macros written in IGOR Pro (Wavemetrics).

2.5 Experimental design and statistical analysis.

TRN neuronal firing evoked by brief (25 ms) current steps was classified into two groups, bursting and persistent firing (PF), based on the duration of the evoked membrane depolarization measured at half-amplitude (see Results). Post-hoc K means cluster analysis confirmed that classification into two clusters explained the largest amount of variance. I used a depolarization duration of 200 ms to distinguish between bursting and PF. Due to rundown of PF in whole-cell mode, data acquisition in the TRN was limited to 3 min following establishment of whole-cell configuration. Pharmacological experiments examining the mechanisms underlying PF were carried out by performing recordings in the presence of a given antagonist under study, and comparing results with recordings performed under control conditions. All data in a given group were collected from slices derived from a minimum of 2 animals and reported as mean ± SEM. All statistical analyses were performed with Prism 7 software. To evaluate significant differences in the proportion of PF between different experimental groups I performed a chi square test, and report results in the following format: degrees of freedom in parentheses, followed by the *chi-square* value and *p* value. To test for significant differences in the duration of evoked membrane depolarization between groups of neurons, I performed an unpaired *t*-test and report results as: degrees of freedom in parentheses, followed by *t* value and *p* value. Statistical significance was set at $p < 0.05$ and adjusted for multiple statistical comparisons with a Bonferroni correction to correct for pairwise error and to reduce Type I error

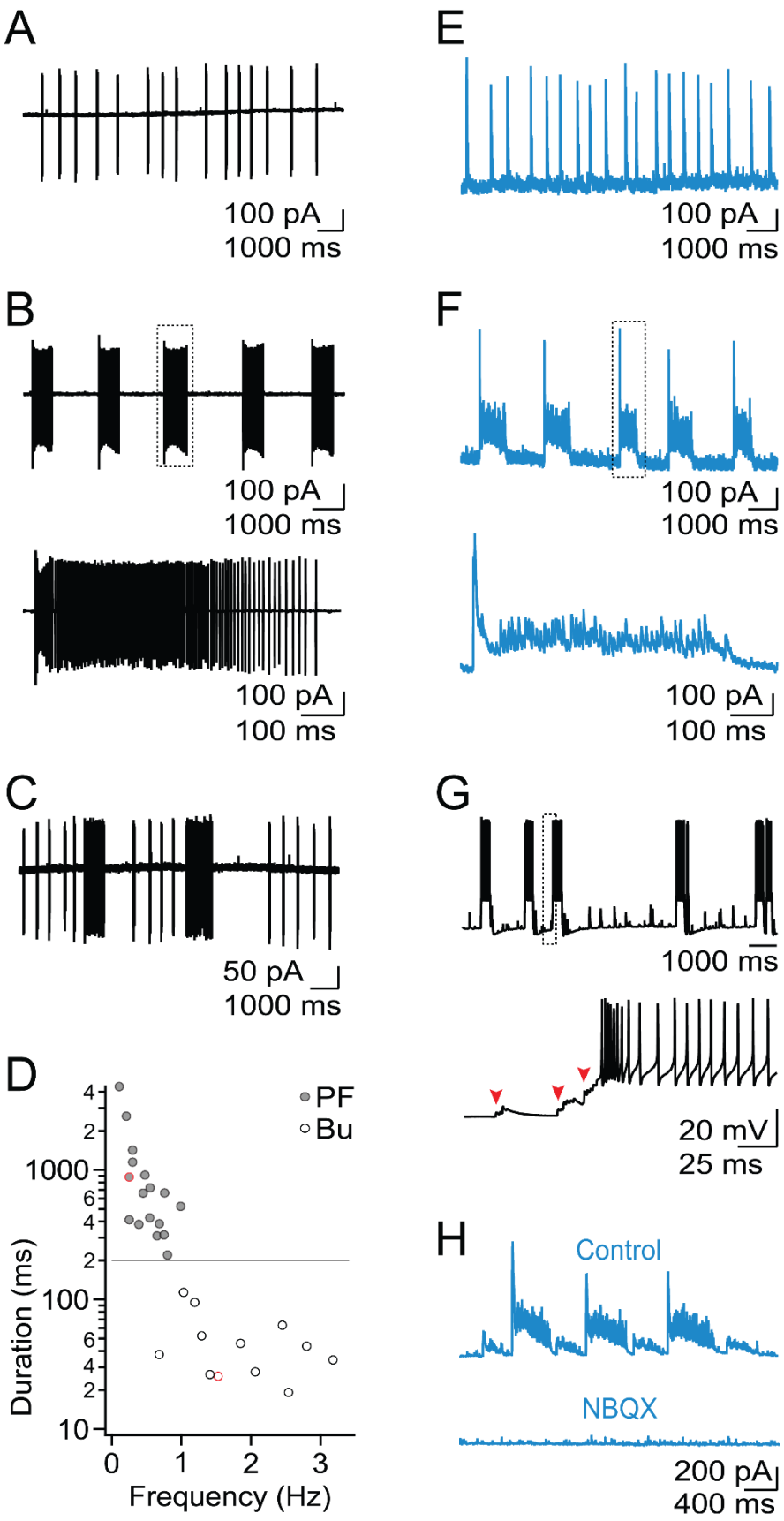
Chapter 3: Results

Reproduced with permission from O'Malley, J.J., Seibt, F., Chin, J., & Beierlein, M. (2020) TRPM4 conductances in thalamic reticular nucleus neurons generate persistent firing during slow oscillations. *J. Neurosci.* 40(25), doi: 10.1523/jneurosci.0324-20.2020. (O'Malley et al., 2020)

3.1 Slow oscillatory activity and persistent firing (PF) in thalamic networks

Following activation of postsynaptic Group I mGluRs, individual neurons in the TRN can generate slow oscillatory responses, reminiscent of thalamic activity during sleep (Blethyn et al., 2006). It remains unclear how thalamic synaptic networks contribute to slow oscillatory activity. To examine this issue, I performed experiments in horizontal thalamic slices derived from adult (> 3 month) mice. I and others have previously employed extracellular or optogenetic synaptic stimulation to trigger transient (< 2 s) forms of rhythmic thalamic activity *in vitro* (Huguenard and Prince, 1994; Pita-Almenar et al., 2014b). Surprisingly, by performing loose-patch recordings from TRN neurons, I observed spontaneous and highly rhythmic patterns of action potential activity that could last several minutes (n = 47 TRN neurons, n = 17 slices). For 12/47 (26%) of recordings, action potential activity was organized as rhythmic bursting (frequency: 1.9 ± 0.1 Hz, n = 12, Fig. 3.1A). Interestingly, a second group of recordings (17/47, 36%) revealed rhythmic patterns of sustained action potential activity (> 200 ms duration) I termed persistent firing (PF), characterized by an initial high-frequency burst, followed by a long-lasting train of action potentials (average duration: 964.9±256.4 ms, average firing frequency: 175±23.4 Hz, n = 17, Fig. 3.1B). For neurons displaying rhythmic PF, oscillation frequency was 0.5 ± 0.1 Hz (n = 17), significantly lower compared to the oscillation frequency of cells showing burst firing ($t(28) = 6.5$, $p = 10^{-16}$,Fig. 3.1D).

Figure 3.1. Rhythmic activity in thalamic networks. A. Loose-patch recording of TRN neuron showing rhythmic burst firing. **B.** *Top,* TRN neuron showing rhythmic persistent firing (PF). *Bottom*, Close-up highlighting an individual barrage of PF. **C.** TRN neuron showing rhythmic activity consisting of both burst firing and PF. **D.** Summary data (n = 29) plotting the average duration of TRN firing as a function of oscillation frequency, for neurons with either burst firing or PF. Data points in red denote cells shown in A and B. Dashed line marks criterion separating between rhythmic bursting and PF. **E.** Voltage-clamp recording of VB neuron held at 0 mV displaying rhythmic bursts of IPSCs. **F.** *Top,* Recording of VB neuron displaying rhythmic barrages of IPSCs. *Bottom*, close-up shows an individual barrage of IPSCs. **G.** *Top*, Current clamp recording of TRN neuron, showing PF during network activity. *Bottom*, Close up shows that bursts of EPSCs (marked by red arrows) trigger PF. **H.** Representative recording of rhythmic barrages of IPSCs in VB neuron, under control conditions and following bath application of NBQX (10 μM). Similar data were obtained from 4 other VB neurons.


A
100 pA
1000 ms
B
100 pA
1000 ms
100 pA
100 ms
C
50 pA
1000 ms
D
PF
Bu
Duration (ms)
Frequency (Hz)
E
100 pA
1000 ms
F
100 pA
1000 ms
100 pA
100 ms
G
1000 ms
20 mV
25 ms
H
Control
NBQX
200 pA
400 ms

The remaining recordings (n=18) showed more complex patterns of activity, and included neurons that displayed both rhythmic PF and burst firing (n=4, Fig. 3.1C). These data indicate that burst firing and PF are prominent firing modes in the TRN and are associated with oscillatory activity at distinct frequencies.

Relay neurons in the ventrobasal nucleus (VB) receive their sole inhibitory input from neurons in the TRN. Therefore, the patterns of synaptic inhibitory responses recorded in VB neurons should closely reflect rhythmic spike firing observed in TRN. To confirm this, I performed voltage-clamp recordings from VB neurons at a holding potential of 0 mV to isolate IPSCs. In 76% (n = 22/29) of all VB neurons that showed oscillatory synaptic activity, we recorded synaptic responses organized as long-lasting high-frequency IPSC barrages (duration: 620.1±54.3 ms, n = 32, Fig. 3.1F) which occurred rhythmically over several minutes (0.5±0.1 Hz, n = 32). A smaller fraction of recordings showed rhythmic IPSC bursts (n = 4, Fig. 3.1E). Thus, relay neurons are the target of persistent inhibition during slow oscillatory activity, likely mediated by TRN neurons with PF.

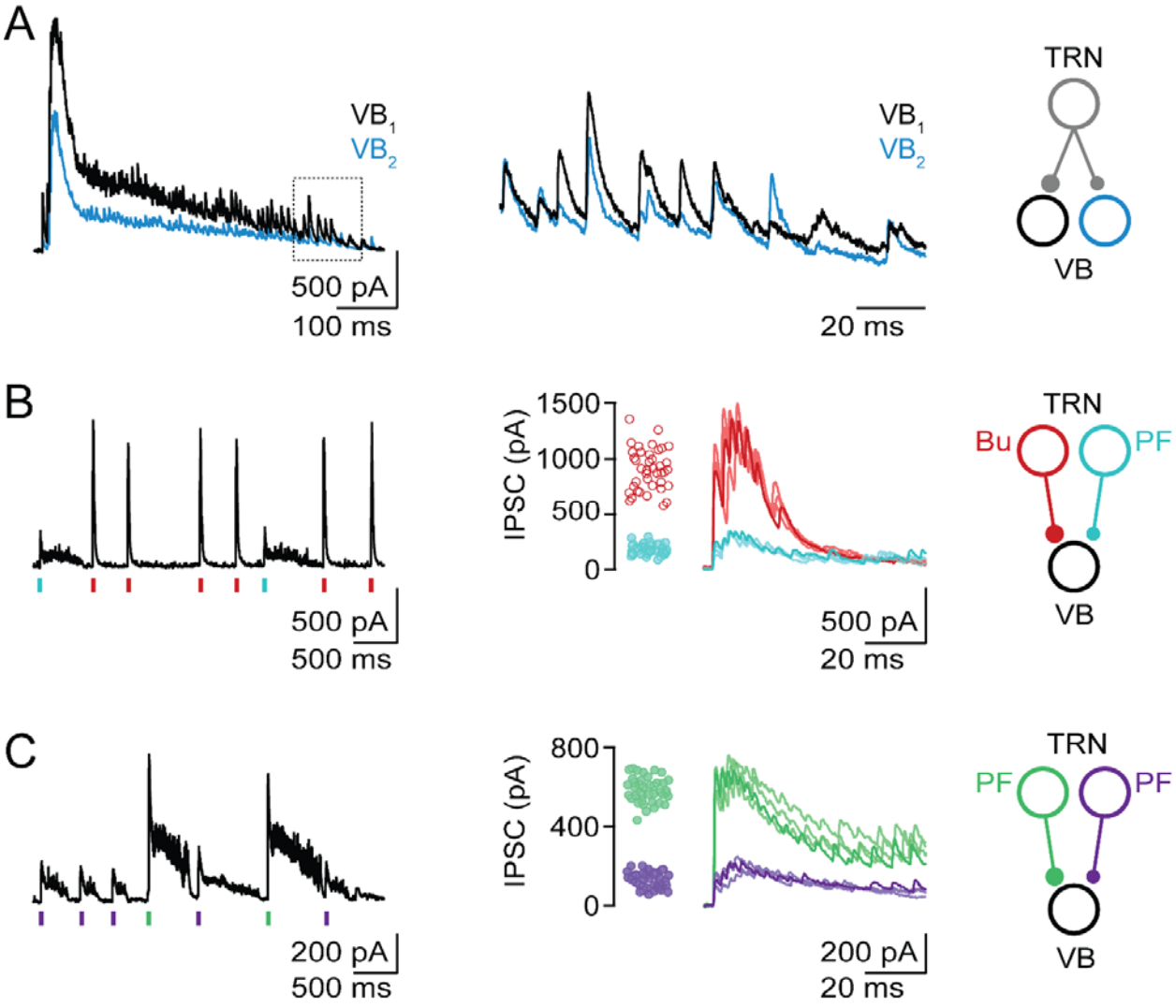
Postsynaptic mGluR activation by exogenous agonists or high-frequency stimulation of glutamatergic afferents can lead to slow oscillatory activity in TRN neurons, even in the absence of fast synaptic signaling (Blethyn et al., 2006). In contrast, by performing whole-cell recordings in TRN, I found that rhythmic patterns of PF did not occur spontaneously but instead were evoked by brief bursts of large-amplitude thalamoreticular EPSPs (Fig. 3.1G). Furthermore, rhythmic IPSCs recorded in VB cells were fully blocked by bath application of NBQX (n = 5, Fig. 3.1H), suggesting that similar to spindle-like activity (Huguenard and McCormick, 2007), slow oscillatory activity relied on a network of interconnected TRN and relay neurons, at least under my experimental conditions.

Anatomic and functional studies have shown considerable axonal divergence for TRN to VB projections, resulting in highly overlapping TRN afferents to neighboring VB neurons (Pinault, 2004; Pita-Almenar et al., 2014b). In agreement, the timing and duration of oscillatory IPSC barrages were highly synchronous for local VB neuronal pairs (< 50 μm somatic distance) recorded simultaneously (Fig. 3.2A). Synchrony was maintained for individual IPSCs throughout the entire barrage suggesting that the large majority of IPSCs were spike-mediated, without a detectable contribution of asynchronous GABA release (Hefft & Jonas, 2005).

I found that the majority of VB recordings showed oscillatory IPSC events with little variability of the initial IPSC amplitude and event duration (Fig. 3.1E,F), consistent with a single presynaptic TRN neuron. However, for a number of recordings I observed either two (n=10 VB cells, Fig. 3.1H and 3.2B,C) or three (n=4 VB cells, *data not shown*) different types of rhythmic IPSC events, with each type characterized by a distinct initial IPSC amplitude and event duration, suggesting convergent inputs from two or three presynaptic TRN neurons, respectively. For recordings with two types of events, event duration suggested convergent input by TRN cells that either both displayed PF (n=6, Fig. 3.2C) or PF and bursting (n=4, Fig. 3.2B). Importantly, IPSC events evoked by different presynaptic TRN neurons displayed clear rhythmicity but never occurred synchronously. These data confirm my previous findings derived in thalamic slices of less mature animals (Pita-Almenar et al., 2014b) and suggest that local thalamic circuits can mediate ongoing oscillatory activity in the absence of widespread synchrony.

To further probe the lack of synchrony of TRN cells during rhythmic activity I performed dual loose patch recordings of neighboring TRN cells (< 50 μm somatic distance).

Figure 3.2. Local TRN neurons display rhythmicity in the absence of precise synchrony. VB recordings were performed in voltage clamp, at a holding potential of 0 mV. **A.** Divergent TRN output to VB neurons. *Left*, Dual recordings of neighboring VB cells showing a single barrage of IPSCs. *Middle*, Close-up of IPSCs (normalized to the initial IPSC amplitude) highlighting precise IPSC synchrony across both cells. *Right*, Schematic outlines proposed divergent TRN projections giving rise to VB responses. **B.** TRN neurons with PF and burst firing form convergent input onto VB neuron**.** *Left,* Voltage-clamp recording of VB neuron with IPSC bursts (red tick marks) and prolonged IPSC barrages (cyan tick marks) with distinct initial amplitudes. *Middle*, For the same recording, overlay of representative IPSC events and summary data (n=40 for each event) quantifying the initial amplitude of IPSC events**.** *Right*, Schematic of proposed circuit. **C.** Two TRN neurons with PF form convergent input onto VB neuron**.** *Left*, Recording of VB neuron showing IPSC barrages with two distinct initial amplitudes (green and purple tick marks, respectively). *Middle*, For the same recording, overlay of representative IPSC events and summary data (n=40 for each event) quantifying the initial amplitude of IPSC events. *Right*, Schematic of proposed circuit.


A
VB1
VB2
500 pA
100 ms
VB1
VB2
20 ms
TRN
VB
B
500 pA
500 ms
IPSC (pA)
1500
1000
500
0
500 pA
20 ms
TRN
Bu
PF
VB
C
200 pA
500 ms
IPSC (pA)
800
400
0
200 pA
20 ms
TRN
PF
PF
VB

For 11/14 paired recordings, rhythmic activity (PF, n=6 or bursting, n=5) was limited to one cell while the other neuron remained silent (Figure 3.3A,B). The remaining three pairs had activity in both cells, but firing was never synchronous. (Figure 3.3C). Taken together these data, in conjunction with my VB recordings, suggest that there is little synchrony of slow oscillations between TRN neurons.

3.2 Brief depolarizations trigger PF in TRN neurons

So far my data indicate that a significant fraction of TRN neurons shows PF during network activity. To better characterize the properties and underlying mechanisms of PF, I performed whole-cell recordings and blocked network activity by adding NBQX (10 μM) to the bath solution. Neurons were held at membrane potential of -75 mV unless stated otherwise and actions potentials were evoked using brief depolarizing current steps (25 ms, 400 pA). Such stimuli led to bursts of action potentials (average number of spikes: 8.1±0.2, n = 74) in 67% of neurons recorded (Fig. 3.4A). For the remaining neurons (37/111, 33%) I observed sustained action potential activity (average number of spikes: 72.2±9.8, firing frequency: 99.1±7.5 Hz, n = 37) closely resembling PF observed during network activity. PF was mediated by long-lasting plateau potentials (amplitude from rest: 29.3±0.8 mV, plateau duration: 807.1±41.7 ms, n = 37, Fig. 3.4A,B) that terminated in a stepwise manner. Using the duration of the current-step evoked membrane depolarization, I performed K means cluster analysis, confirming that TRN firing patterns can be most readily classified into two distinct groups, bursting and PF, with the latter group displaying membrane depolarizations of > 200 ms duration. Neurons with current-step evoked PF were not restricted to a specific TRN subregion and were localized along the entire thickness of the TRN shell (not shown).

Figure 3.3 Lack of synchrony in TRN neurons during spontaneous network activity. Dual loose patch recordings of TRN neurons during spontaneous network activity. **A.** Paired recording with one cell showing burst firing (*top*) and the other cell displaying no activity (*bottom*). **B.** Another paired recording with one cell exhibiting PF (*top*) and the other cell displaying no activity (*bottom*). **C.** Paired recording with both cells exhibiting PF, in the absence of synchrony.

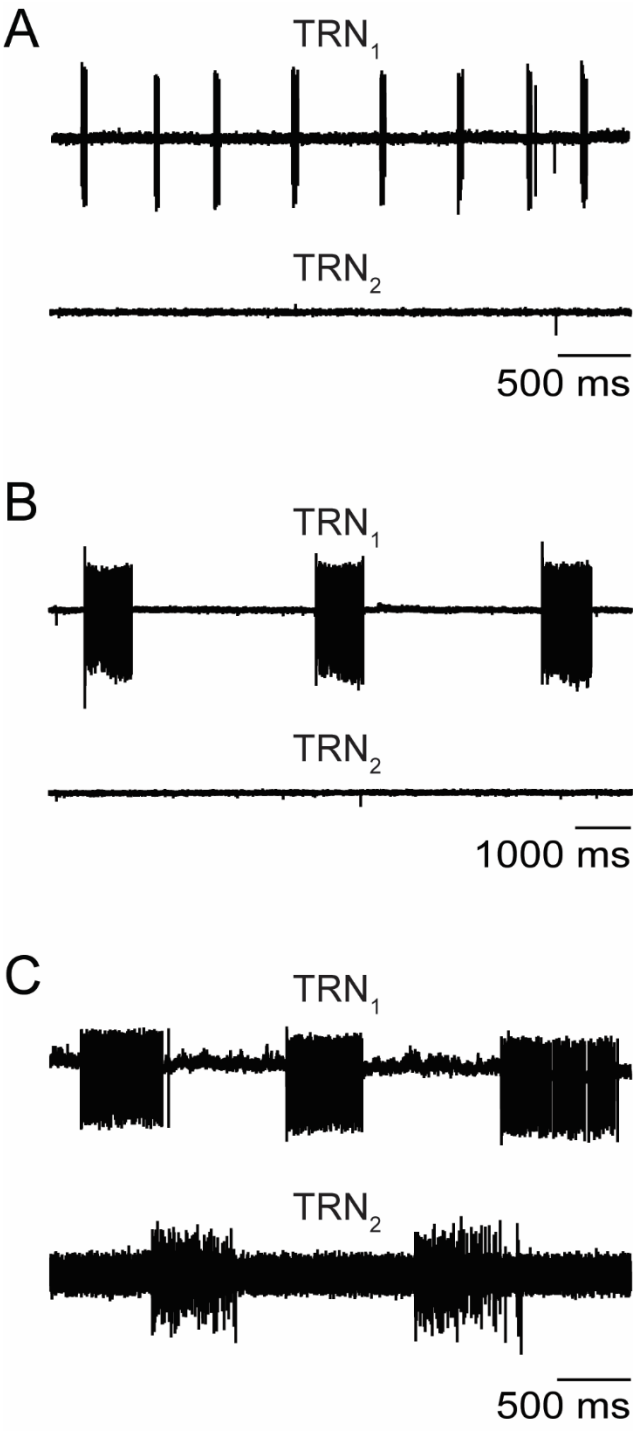
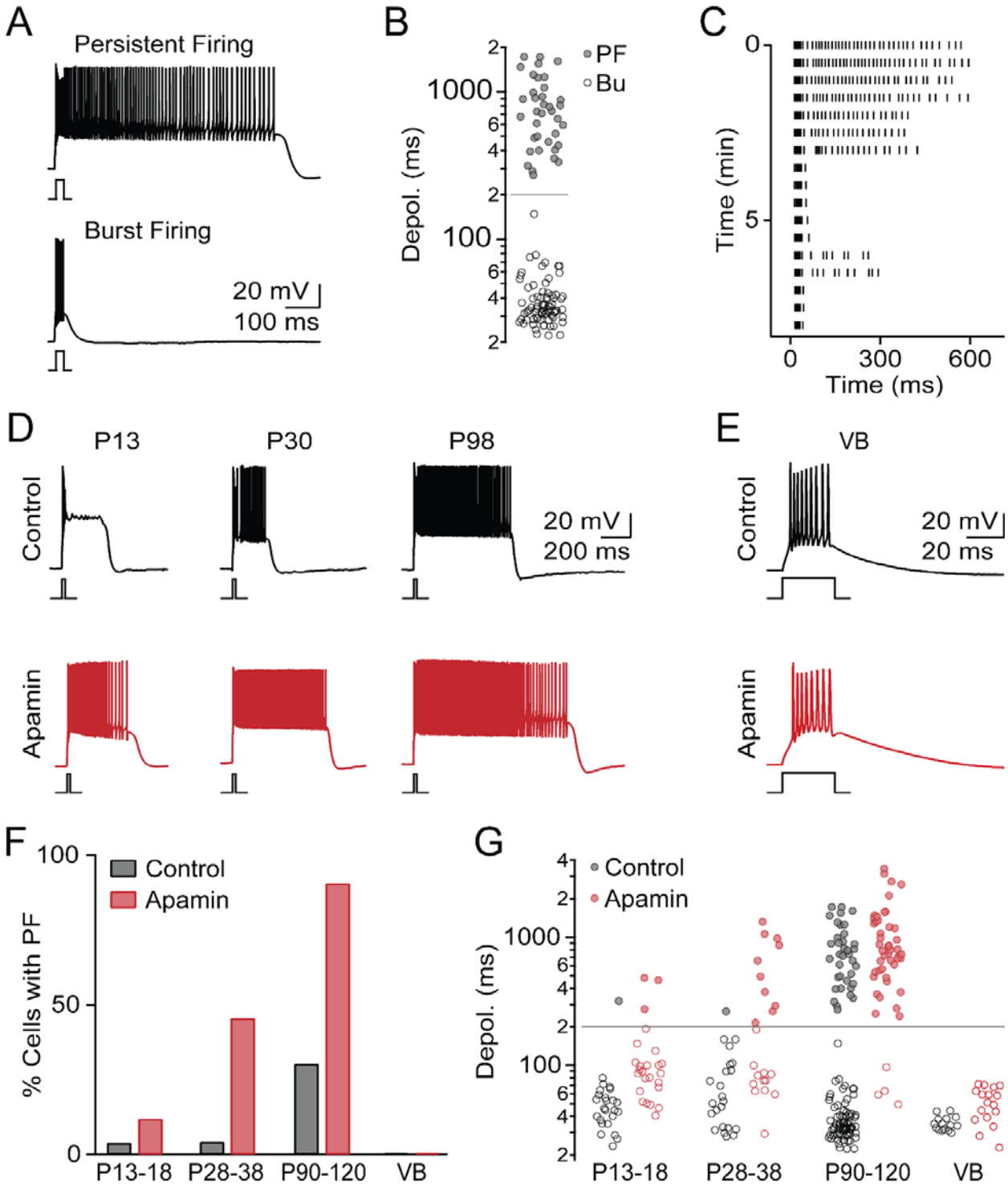

A
TRN1
TRN2
500 ms
B
TRN1
TRN2
1000 ms
C
TRN1
TRN2
500 ms

Figure 3.4. PF occurs late in development and regulated by SK conductances. A. Brief depolarizing current steps (25 ms, 400 pA, RMP = -75 mV) lead to two distinct firing patterns, Persistent Firing (PF, *top*) or Burst Firing (Bu, *bottom*). **B.** Summary data (n = 111) showing bimodal distribution of current-step evoked membrane depolarizations. Grey line indicates criterion separating PF and Bu. **C.** Raster plot of AP activity in representative TRN neuron showing rundown of PF. **D.** Representative recordings of TRN neurons with PF at three developmental stages, in control conditions (*black*) and in the presence of apamin (500 nM, *red*). **D.** Representative recordings of TRN neurons with PF at three developmental stages, in control (black) and in the presence of apamin (500 nM, *red*). **E.** Representative recordings of VB cells, in control (black) and in the presence of apamin (red) show lack of PF in both conditions. **F.** Summary data showing incidence of PF in TRN across development (P13-18: n = 24 in control, n = 25 in apamin, P28-38: n = 23 in control, n = 22 in apamin, P90-120: n = 111 in control, n = 50 in apamin) and in VB (n = 15 in control, n = 16 in apamin). **G.** For the same cells as in F, summary data quantifying membrane potential duration for all TRN cells across development, in control conditions and in apamin.


A
Persistent Firing
Burst Firing
20 mV
100 ms
B
PF
Bu
Depol. (ms)
1000
100
C
Time (min)
0
5
0
300
600
Time (ms)
D
P13
P30
P98
Control
Apamin
20 mV
200 ms
E
VB
Control
Apamin
20 mV
20 ms
F
% Cells with PF
100
50
0
Control
Apamin
P13-18
P28-38
P90-120
VB
G
Control
Apamin
Depol. (ms)
1000
100
P13-18
P28-38
P90-120
VB

I found that cells displaying PF and bursting showed similar active properties (Table 3.1), suggesting that these firing patterns are not associated with distinct cell types. PF remained stable for several minutes but then decayed over time, regardless of composition of the internal solution (Fig. 3.4C). This might partly explain why studies employing whole-cell methods have not reported PF in adult TRN neurons (e.g. Clemente-Perez et al., 2017), while at least one study using sharp electrode recordings described PF in a subset of neurons of the cat perigeniculate nucleus (Kim & Mccormick, 1998). For the remainder of this study I limited data acquisition to the first 3 minutes in whole-cell mode.

3.3 PF occurs late during development and is controlled by SK conductances

Previous work characterizing TRN firing patterns in slices derived from younger (<P30) animals has not reported PF (Lee et al., 2007), suggesting that PF is developmentally regulated. To examine this possibility, I carried out additional recordings in slices prepared from mice aged P13-18 and P28-38. Compared to adult mice, only 1/24 (4%) of cells at P13-18 and 1/23 (4%) of cells at P28-38 displayed PF (Fig. 3.4D-F), indicating that PF is prominent only in fully mature animals.

The generation of plateau potentials that underlie PF is likely to be tightly regulated by K^+ conductances, including small conductance Ca^{2+}-activated K^+ channels (SK). To examine this possibility, I performed recordings in the presence of the SK channel blocker apamin (100 nM). Under these conditions, TRN firing rates significantly increased (Table 3.2) and a much larger fraction of TRN cells in adult animals showed PF (92%, n = 46/50 $\chi^2(1) = 47.5$, $p = 10^{-16}$, Fig. 3.4D-F). Thus, excitatory conductances responsible for PF are widely expressed in the adult TRN but appear to be strongly controlled by SK conductances.

	Bursting	PF	*P*
R_{IN} (MΩ)	169.38 ± 15.15	127.27 ± 11.89	0.1
Tau (ms)	10.54 ± 0.85	9.67 ± 0.78	0.54
Latency to First Spike (ms)	3.85 ± 0.35	5.01 ± 0.38	0.06
# of Spikes	7.93 ± 0.3	9.06 ± 0.33	0.06
Spike Width (ms)	0.27 ± 0.01	0.27 ± 0.01	0.99
Peak Firing Freq. (Hz)	400.7 ± 18.94	496.0 ± 20.92	0.005

Table 3.1 Passive and active properties of adult TRN neurons. All data presented as Mean ± SEM, *p* values reported are based on unpaired *t*-tests comparing Bursting and PF. Membrane input resistance (R_{IN}) was calculated from the membrane potential during a hyperpolarizing current step divided by the current applied. The membrane time constant tau was calculated by fitting an exponential curve to the hyperpolarization. Active properties were measured during the depolarizing current step.

Given the role of SK channels in regulating PF in the adult, the developmental increase in PF I observed might be solely mediated by a progressive decrease of SK expression. However, I found that even in the presence of apamin the incidence of PF remained very low early in development and dramatically increased with animal age (P13-18: 12%, n = 25, P28-38: 41%, n = 24, P90-120: 92%, Fig. 3.4D-F), suggesting a developmental upregulation of excitatory mechanisms that underlie PF. Finally, I examined if neurons in the VB displayed plateau potentials in control or in the presence of apamin. In contrast to TRN, PF was completely absent from VB under both conditions (Fig. 3.4E,G). Thus, the mechanisms underlying PF do not appear to be uniformly expressed in all thalamic nuclei.

3.4 Metabotropic glutamate receptor activation is not necessary for PF

Previous work has highlighted a critical role of postsynaptic group I mGluR activation in enabling intrinsic oscillations and Up states in TRN neurons (Blethyn et al., 2006). While PF under my experimental conditions did not require exogenous or synaptic mGluR I activation, it is possible that mGluRs were tonically activated by ambient glutamate (Crabtree et al., 2013) or in a ligand independent manner (Sun et al., 2016). To examine this possibility, I probed TRN firing in the presence of the non-competitive mGluR1 antagonist JNJ 16259685 (JNJ) and the non-competitive mGluR5 antagonist MTEP. Block of group I mGluRs did not significantly change the incidence of PF (control: 33%, n = 111, JNJ+MTEP: 48%, n = 27, $\chi^2(1) = 1.7$, $p = 0.19$), the plateau potential duration for cells with PF (control: 807.1±41.7 ms, JNJ+MTEP: 599.8±64.3 ms, $t(46) = 1.85$, $p = 0.071$, Fig. 3.5A,B), or the active properties (Table 3.2). These data suggest that under my experimental conditions Group I mGluR activation is not essential for PF.

	Latency (ms)	# of Spikes	Spike Width (ms)	Peak Freq. (Hz)
[&]Control	4.19 ± 0.28	8.26 ± 0.27	0.27 ± 0.01	428.64 ± 15.92
MTEP+JNJ	4.12 ± 0.29	9.0 ± 0.22	0.28 ± 0.01	479.53 ± 12.46
Apamin	3.86 ± 0.24	10.04 ± 0.28^{++}	0.32 ± 0.01^{++}	489.41 ± 12.29^{++}
+ TTA-P2	5.74 ± 0.88*	6.12 ± 0.46*	0.27 ± 0.02*	311.06 ± 14.6*
+ FFA	6.9 ± 0.64*	5.81 ± 0.46*	0.28 ± 0.01	340.14 ± 16.58*
+ 2-APB	3.66 ± 0.34	10.6 ± 0.31	0.33 ± 0.02	513.5 ± 15.26
+ 9-Phen	4.96 ± 0.47	6.33 ± 0.64*	0.37 ± 0.04	404.01 ± 17.91*
+Glib	6.29 ± 0.49*	7.95 ± 0.33*	0.26 ± 0.01*	442.59 ± 11.16
+ CBA	5.46 ± 0.76*	7.4 ± 0.47*	0.28 ± 0.02*	391.2 ± 24.64*
+ Ba2+	3.45 ± 0.67	8.55 ± 0.62	0.37 ± 0.03	528.31 ± 51.8

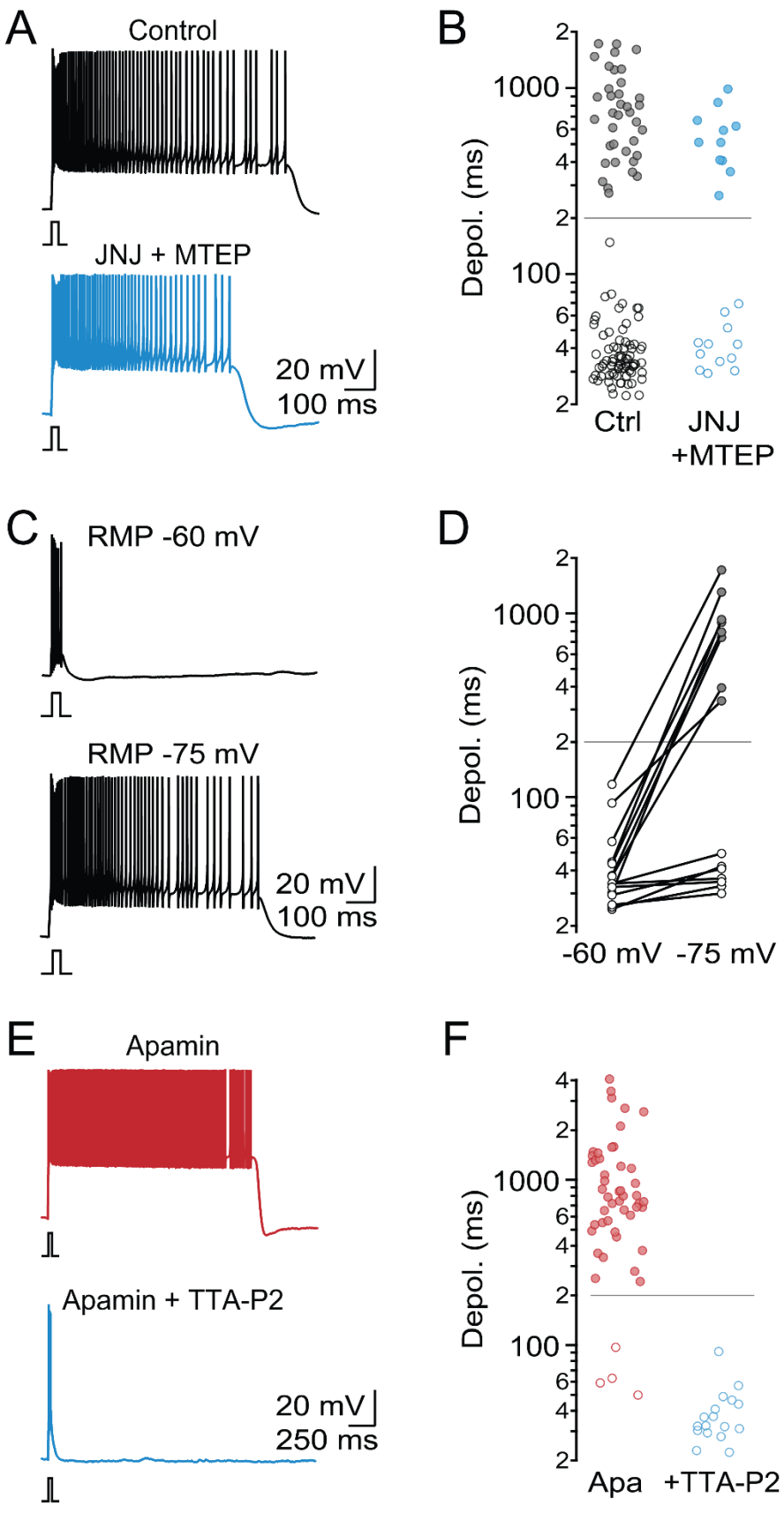
Table 3.2 Active properties of TRN neurons in the presence of pharmacological blockers. All data are reported as Mean ± SEM. Active properties were measured only during the depolarizing current step. All statistical comparisons were made using an unpaired *t*-test and significance was set to $p < 0.05$.

[&] includes both PF and Bursting cells from Table 3.1 to allow comparison to apamin and the Group I mGluR antagonists.

[++] significant compared to cells in control

[*] significant compared to cells in apamin

Figure 3.5. T-type Ca^{2+} channels but not mGluRs are required for PF. A. Representative recordings of PF from two different TRN neurons, in control conditions and in the presence of the mGluR1 antagonist JNJ 16259685 (1 μM) and the mGluR5 antagonist MTEP (10 μM). **B.** Summary data quantifying duration of evoked membrane depolarization in control (n = 111) and in the presence of mGluR 1/5 antagonists (n = 23). **C.** Representative recording of neuron with PF, initially held at a holding potential of -60 mV and then at -75 mV. **D.** Summary data quantifying evoked depolarization duration as a function of holding potential (n = 15 TRN neurons). **E.** Representative recordings in the presence of apamin and in the presence of apamin and the T-type Ca^{2+} channel antagonist TTA-P2 (1 μM). **F.** Summary data quantifying evoked depolarization duration (n = 50 in apamin, n = 17 in apamin + TTA-P2).


A
Control
JNJ + MTEP
20 mV
100 ms
B
Depol. (ms)
1000
100
Ctrl
JNJ
+MTEP
C
RMP -60 mV
RMP -75 mV
20 mV
100 ms
D
Depol. (ms)
1000
100
-60 mV
-75 mV
E
Apamin
Apamin + TTA-P2
20 mV
250 ms
F
Depol. (ms)
1000
100
Apa
+TTA-P2

3.5 Activation of T-type Ca^{2+} channels is required for PF

Next, I examined if PF is voltage-dependent, by evoking action potential activity from two distinct holding potentials. TRN cells held initially at -60 mV never displayed PF or plateau potentials (0/15 cells, Fig. 3.5C,D). However, when the holding potential was adjusted to -75 mV, 8/15 neurons generated PF ($\chi^2(1) = 1.7$, $p = 0.0013$), suggesting that the underlying mechanisms of PF are voltage dependent. To further isolate the mechanisms responsible for PF, I employed a pharmacological approach. Due to the washout of PF in whole-cell mode, it was not possible to quantify changes in PF prior to and following bath application of antagonists for a given neuron. Therefore, I performed all recordings in the presence of a given antagonist and made comparisons to neurons recorded under control conditions (see section 2 *Materials and Methods*). To limit the number of recordings required for meaningful statistical comparisons, I performed the following experiments in the presence of apamin, which, as described above, results in PF in 92% of neurons (Fig. 3.4E,F).

Previous studies have indicated that in thalamic neurons T-type Ca^{2+} channels are critical for the generation of mGluR-dependent Up states (Hughes et al., 2002; Blethyn et al., 2006). The voltage-dependence of PF I observed appears to be consistent with these results. To confirm the involvement of T-type Ca^{2+} channels in generating PF, I performed recordings in the presence of the specific antagonist TTA-P2 (1 μM; see Table 3.2 for active properties). Under these conditions, PF was completely eliminated (apamin: 92%, apamin+TTA-P2: 0%, n = 17, $\chi^2(1) = 46.5$, $p = 10^{-20}$, Fig. 3.5E,F). Thus, T-type Ca^{2+} channels are required for the generation of PF.

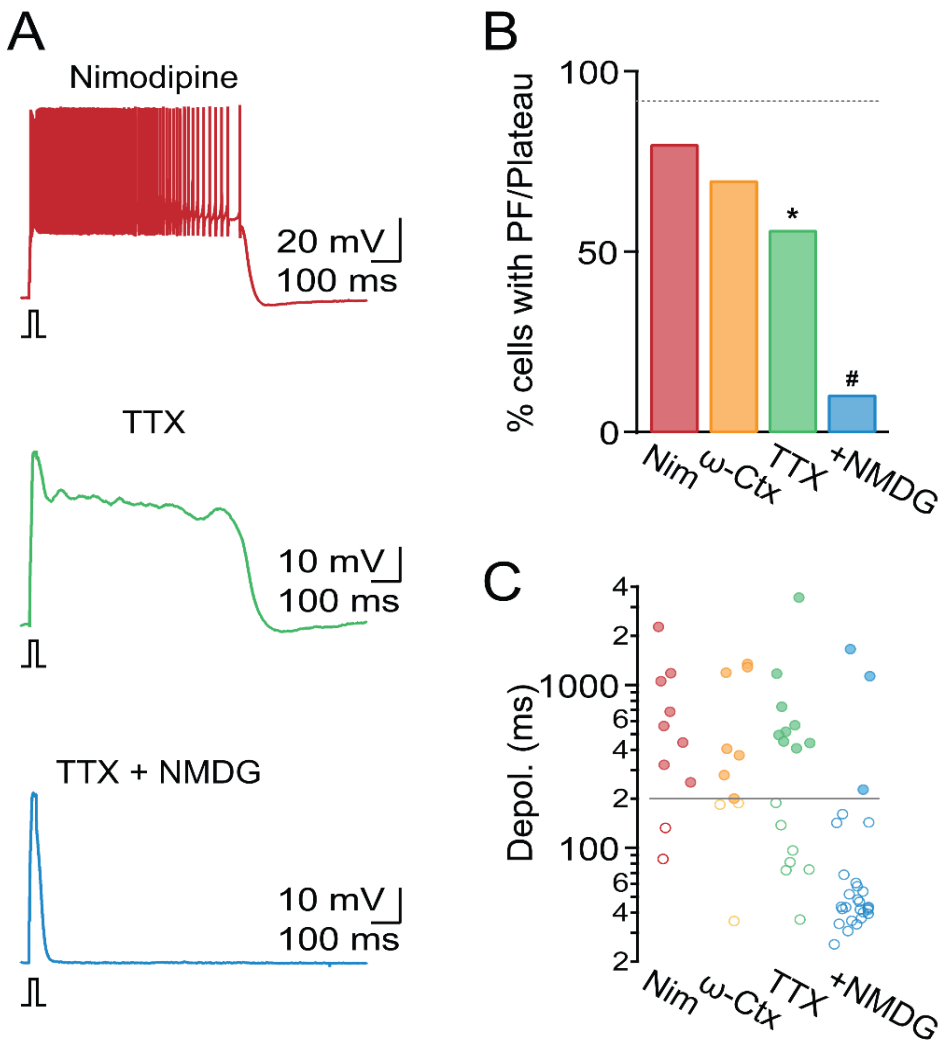
3.6 The plateau potential underlying PF is mediated by a sodium current

It is possible that long-lasting plateau potentials in TRN neurons are entirely mediated by T-type Ca^{2+} conductances (Zylberberg & Strowbridge, 2017). While these channels show rapid inactivation following strong depolarization, their inactivation is not complete for smaller

depolarizations near the resting membrane potential, resulting in a steady-state inward ("window") current (Williams, Tóth, Turner, Hughes, & Crunelli, 1997). However, PF observed in the present study was accompanied by large membrane depolarizations that do not appear to be compatible with a significant role of T-type Ca^{2+} channels. Therefore, I systematically examined the possible involvement of other voltage- or Ca^{2+}-gated non-inactivating conductances. I first tested the role of high-threshold Ca^{2+} channels (L, P/Q, and N). In the presence of the selective L-type antagonist nimodipine (nim, 20 μM, Fig. 3.5A) the incidence of PF (apamin: 92%, apamin+Nim: 80%, n = 10, $\chi^2(1) = 0.3$, $p = 0.6$, Fig. 3.6B) and plateau potential duration for cells with PF (apamin: 1108.5±126.6 ms, apamin+Nim: 846.8±235 ms, $t(48) = 0.9$, $p = 0.38$, Fig. 3.6C) remained comparable to responses recorded in apamin (See Table 3.2 for active properties). Similarly, incubating slices in the P/Q and N antagonist ω-conotoxin MVIIC (ω-Ctx , 1 μM) did not lead to significant changes in the incidence of PF (apamin: 92%, apamin+ω-Ctx: 70%, n = 10, $\chi^2(1) = 1.4$, $p = 0.23$, Fig. 3.6B), and plateau potential duration (apamin: 1108.5±126.6 ms, apamin+ω-Ctx: 726.7±196.2 ms, $t(47) = 1.2$, $p = 0.24$, Fig. 3.6C), suggesting that high-threshold Ca^{2+} channels do not play a critical role in PF.

It is possible that plateau potentials are mediated by activation of a persistent Na^+ current (I_{NaP}) as was suggested previously (Fuentealba, et al., 2005b; Kim & Mccormick, 1998). I found that in the presence of apamin and TTX (500 nM), fast action potential activity was completely blocked, revealing a plateau potential in 9/16 cells (amplitude: 30±1.2 mV, n = 16, Fig. 3.6A), a smaller fraction compared to conditions in apamin (apamin: 92%, apamin+TTX: 56%, $\chi^2(1) = 10$, $p = 0.002$, Fig. 3.6B). Plateau potential duration remained comparable to control (apamin: 1108.5±126.6 ms, apamin+TTX: 912.8±325.7 ms, $t(49) = 0.7$, $p = 0.5$, Fig. 3.6C). These data indicate that voltage-gated Na^+ currents facilitate plateau potential generation, but are not strictly required.

Figure 3.6. A TTX-insensitive sodium current generates plateau potential. All recordings were performed in the presence of apamin. **A.** Representative TRN recordings in the presence of the L-type Ca^{2+} channel antagonist nimodipine (20 μM, *top*), in TTX (500 nM, *middle*), and in the presence of TTX and NMDG replacing extracellular Na^{+} (*bottom*). **B.** Summary data showing incidence of PF in the presence of nimodipine (Nim, n = 10), the P/Q- and N-type Ca^{2+} channel antagonist ω-conotoxin MVIIC (ω-Ctx, 1 μM, n = 10), TTX (n = 16), and TTX and NMDG (n = 28). Dashed line indicates incidence of PF in apamin. **C.** Summary data from the same cells as in B quantifying evoked depolarization duration in each condition. Grey solid line represents criterion for PF. * denotes statistical significance of $p < 0.05$ compared to recordings in apamin group, # denotes statistical significance of $p < 0.05$ compared to recordings in TTX.

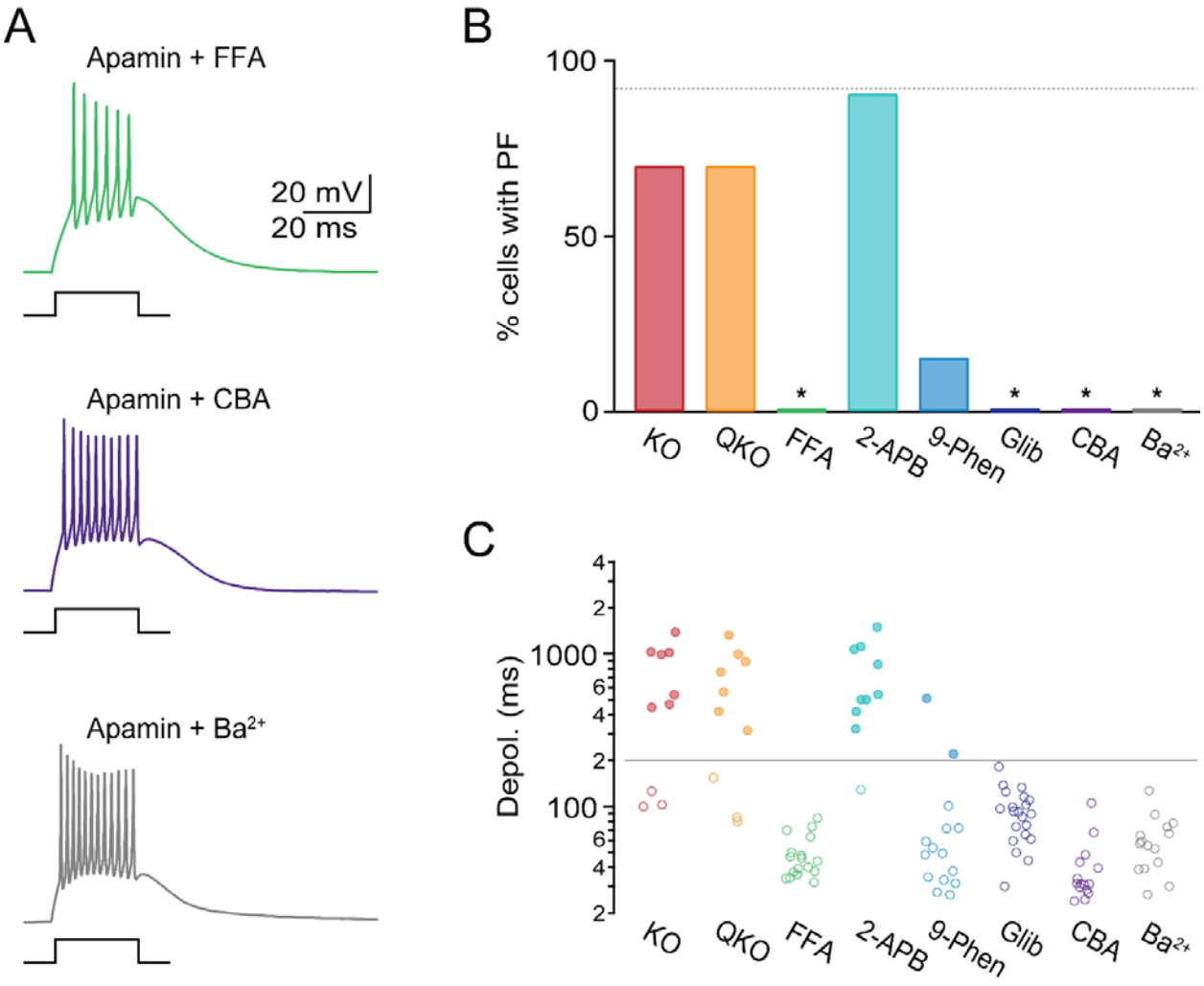

A
Nimodipine
20 mV
100 ms
TTX
10 mV
100 ms
TTX + NMDG
10 mV
100 ms
B
% cells with PF/Plateau
100
50
0
*
#
Nim
ω-Ctx
TTX
+NMDG
C
Depol. (ms)
4
2
1000
6
4
2
100
6
4
2
Nim
ω-Ctx
TTX
+NMDG

Next, I examined if Na^+ currents other than the ones carried by voltage-gated Na^+ channels are essential for plateau potentials, by substituting the majority of extracellular Na^+ with NMDG. I found that in the presence of NMDG (together with apamin and TTX) the incidence of plateau potential generation was significantly reduced (apamin + TTX: 56%, apamin + TTX + NMDG: 11%, n = 28, $\chi^2(1) = 10.6$, $p = 0.001$, Fig. 3.6A-C), suggesting that PF is mediated at least in part by a TTX-insensitive Na^+ current.

3.7 TRPM4 channels underlie PF in the TRN

My data thus far are consistent with a role of the Ca^{2+}-activated nonselective cationic current (I_{CAN}) in mediating plateau potentials. Transient receptor potential (TRP) channels have been previously proposed as molecular mechanisms mediating I_{CAN} (Launay et al., 2002a), with transient receptor potential canonical (TRPC) and melastatin (TRPM) channels as the most likely candidates. To examine the potential involvement of TRPC channels, I performed recordings in slices derived from global TRPC3 null mutant mice (TRPC3 KO, Hartmann et al., 2008) and from global quadruple TRPC1,4,5,6 null mutant mice (QKO, Tian & Zhu, 2018), in the presence of apamin. For both mouse lines, the incidence of PF (apamin: 92%, TRPC3 KO: 70%, n = 10, $\chi^2(1) = 3.4$, $p = 0.65$, QKO: 70%, n = 10, χ^2 (1) = 3.4, p = 0.65, Fig. 3.6B), plateau potential duration (apamin: 1108.5±126.6 ms, TRPC3 KO: 841.1±136 ms, $t(47) = 0.9$, $p = 0.39$, QKO: 752.6±133.4 ms, $t(47) = 1.1$, $p = 0.27$, Fig. 3.7C), and active properties (Table 3.2) were comparable to my observations in WT animals, suggesting that at least 5 of the 7 TRPCs known to be expressed in the brain are not involved in mediating PF.

Figure 3.7. TRPM4 conductances mediate PF. All recordings were performed in the presence of apamin. **A.** Representative recordings in control and in the presence of the non-selective transient potential receptor (TRP) channel antagonist FFA (100 μM, *top*), the TRPM4 antagonist CBA (50 μM, *middle*), and in 2 mM $BaCl_2$ replacing extracellular $CaCl_2$ (*bottom*). **B.** Summary data showing incidence of PF in TRPC3 KO (KO, n = 10 cells) in TRPC1,4,5,6 quadruple KO (QKO, n = 10 cells), FFA (n = 17), the non-selective TRP channel antagonist 2-APB (50 μM, n = 10), the TRPM4/5 antagonists 9-phenthranol (9-Phen, 100 μM, n = 15) and glibenclamide (Glib, 100 μM, n = 21), CBA (n = 15), and Ba^{2+}, (n = 15). Grey dashed line represents incidence of PF in apamin. **C.** For the same cells as in B, summary data quantifying the evoked depolarization duration. * denotes statistical significance of $p < 0.05$ compared to recordings in apamin.


A
Apamin + FFA
20 mV
20 ms
Apamin + CBA
Apamin + Ba2+
B
% cells with PF
100
50
0
KO
QKO
FFA
2-APB
9-Phen
Glib
CBA
Ba2+
C
Depol. (ms)
1000
100
KO
QKO
FFA
2-APB
9-Phen
Glib
CBA
Ba2+

Next, I investigated a possible role of TRPM channels in mediating PF. TRPM2 has been shown to facilitate burst firing (Lee et al., 2013), while both TRPM4 and TRPM5 have been implicated in mediating slow inward currents in cortical cells (Lei et al., 2014) and in cerebellar Purkinje cells (Kim et al., 2013). I found that in the presence of the TRP channel inhibitor FFA (100 μM), PF was completely eliminated (apamin: 92%, apamin+FFA: 0%, n = 17, $\chi^2(1) = 46.6$, $p = 10^{-20}$, Fig. 3.7A-C). The spike latency, number of spikes during the depolarization, and peak firing frequency were reduced in FFA (Table 3.2). Next, I examined the effects of the broad-spectrum inhibitor 2-APB which among TRP channels blocks TRPC7 and TRPM2, but does not inhibit TRPM4 or TRPM5. 2-APB had no effect on PF (apamin: 92%, apamin+ 2-APB: 90%, n = 10, $\chi^2(1) = 0.4$, $p = 0.54$, Fig. 3.7B) or active properties (Table 3.2), with plateau potential duration comparable to control conditions (apamin: 1108.5±126.6 ms, apamin+2-APB: 758±132.4 ms, $t(49) = 1.3$, $p = 0.22$, Fig. 3.7C), ruling out a role of TRPM2 or TRPC7 and suggesting that either TRPM4 or TRPM5 might mediate PF. Both channels are voltage-dependent, monovalent-selective, and require increases in intracellular Ca^{2+} for their activation (Launay et al., 2002; Nilius et al., 2003). I found that in the presence of the TRPM4/5 antagonists 9-Phenthranol (9-Phen, 100 μM) and glibenclamide (Glib, 100 μM) PF was completely eliminated (apamin: 92%, apamin+9-Phen: 15%, n = 15, $\chi^2(1) = 34.2$, $p = 10^{-14}$, Glib: 0%, n = 21, $\chi^2(1) = 51.4$, $p = 10^{-26}$, Fig. 3.7B). Additionally, 9-phen significantly reduced the number of spikes evoked by the current step, and the peak firing frequency (Table 3.2). Glib significantly reduced the latency of spiking, the number of spikes evoked and the action potential width (Table 3.2). Lastly, CBA significantly reduced all active properties (Table 3.2). Next, I performed experiments using the recently developed selective TRPM4 antagonist CBA (Ozhatil et al., 2018). In the presence of CBA (50 μM, see Table 3.2 for active properties), PF was completely

eliminated (apamin: 92%, apamin + CBA: 0%, n = 15, $\chi^2(1) = 44$, $p = 10^{-17}$, Fig. 3.7A,B). Taken together, my results indicate that TRPM4 conductances mediate the plateau potential underlying PF.

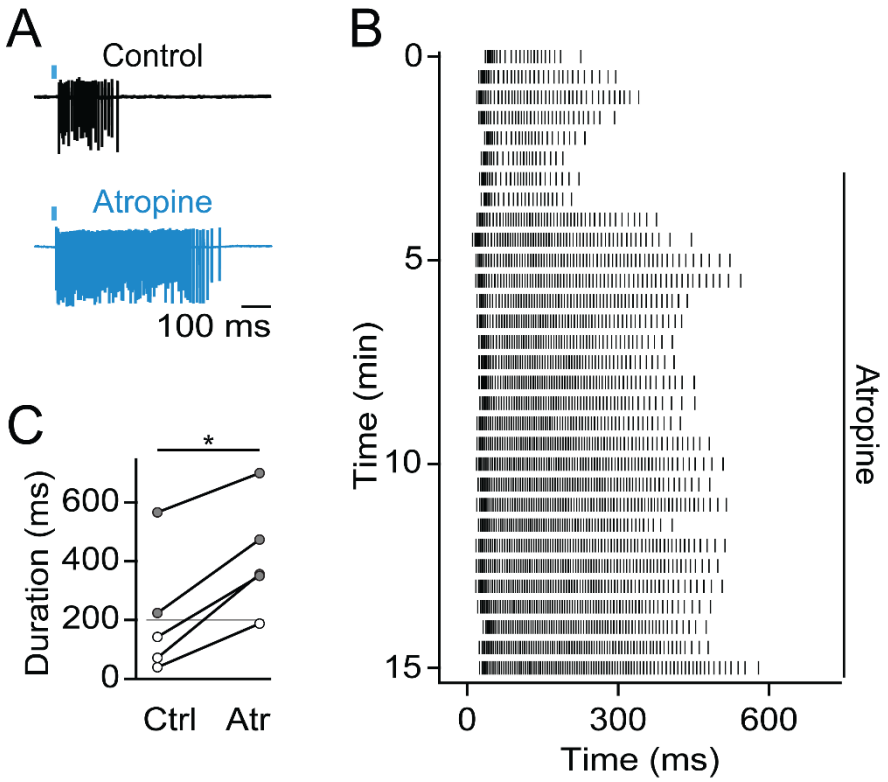
Since increases in intracellular Ca^{2+} are required for TRPM4 activation, exogenous buffers such as BAPTA should eliminate PF. However, due to the rundown of PF, experiments relying on the infusion of Ca^{2+} chelators were not feasible. I therefore probed a possible Ca^{2+} requirement of PF by replacing extracellular Ca^{2+} with equimolar Ba^{2+}. Ba^{2+} passes through T-type Ca^{2+} channels and generates large inward currents (Huguenard & Prince, 1992, see Table 3.2 for active properties), but unlike Ca^{2+} does not lead to TRPM4 activation (Nilius et al., 2004; Yamaguchi et al., 2014). Under these conditions, brief depolarizing steps still led to prominent burst firing, indicating that T-type Ca^{2+} channel dependent dendritic depolarizations were not affected (Fig. 3.7A). However, none of the cells displayed PF (apamin: 92%, apamin+Ba^{2+}: 0%, n = 15, $\chi^2(1) = 44$, $p = 10^{-17}$, Fig. 3.7B,C). These data further confirm that T-type Ca^{2+} channels alone are not sufficient to generate plateau potentials. Instead, my data suggest that T-type Ca^{2+} channel activation and the resulting Ca^{2+} increases lead to the activation of TRPM4 conductances and the generation of PF.

3.8 Synaptic recruitment of muscarinic acetylcholine receptors suppresses PF

Finally, I examined the modulation of PF by synaptic activity. TRN neurons are the target of cholinergic afferents from the basal forebrain and brainstem (Hallanger & Wainer, 1988). My previous studies have shown that acetylcholine (ACh) release leads to a biphasic postsynaptic response, with fast excitation mediated by nicotinic ACh receptors (nAChRs) and slow and long-lasting inhibition mediated by M2 muscarinic receptors (mAChRs) coupled G protein-gated inward rectifying K^+ (GIRK) channels (Sun et al., 2013). To examine if cholinergic inputs can trigger PF in the TRN, I performed experiments using ChAT-ChR2-EYFP mice that selectively express ChR2 in cholinergic neurons (Zhao et al., 2011) and optogenetically evoked ACh release with light pulses (1

ms) centered over the recorded neuron. To avoid decay of PF, TRN neurons were recorded in loose-patch mode. Under these conditions, 2/5 of neurons displayed PF (Fig. 3.8A,C). I reasoned that the activation of mAChRs and the resulting GIRK activation might limit a full manifestation of PF. Indeed, following atropine application to block mAChRs, the duration of action potential firing was significantly prolonged in all neurons recorded (control: 213.5±94 ms, atropine: 416.3±84.1 ms, $t(4) = 7.1$, $p = 0.002$, Fig. 3.8A-C), and the incidence of PF increased from 40% to 80%. These data show that the activation of K^+ channels by synaptically released neuromodulators such as ACh can dynamically modulate the expression of PF.

Figure 3.8. Synaptic recruitment of muscarinic AChRs suppresses PF. Recordings were carried out in slices of ChAT-ChR2-EYFP BAC mice. **A.** Loose-patch recording of a TRN neuron showing brief barrage of ACh-evoked activity (*black*), which is prolonged following application of the mAChR antagonist atropine (10 μM, *blue*). **B.** Raster plot of ACh-evoked neuronal activity prior to and following atropine application, for the cell shown in A. **C.** Summary data quantifying the average duration of ACh-evoked action potential activity before and after atropine application (n = 5). * $p < 0.05$.


A
Control
Atropine
100 ms
B
0
5
10
15
Time (min)
Atropine
0
300
600
Time (ms)
C
*
Duration (ms)
600
400
200
0
Ctrl
Atr

Chapter 4: Discussion and Conclusions

Here I have identified TRPM4 as the mechanism underlying PF in adult neurons of the TRN. My data indicate that the large depolarizations and intracellular Ca^{2+} increases required for TRPM4 activation are mediated by T-type Ca^{2+} channels. Additionally, I found that TRPM4 mediated PF can be modulated through cell intrinsic mechanisms, such as SK channels and mAChR-activated GIRK channels. PF was associated with robust slow oscillatory activity in thalamic circuits which required fast synaptic transmission but did not rely on mGluR receptor signaling. Previous studies have shown that both TRN and thalamic relay neurons can act as cellular pacemakers for slow thalamic oscillations. My present findings highlight intrathalamic circuits as an alternative mechanism capable of generating slow rhythmic activity.

4.1 Mechanisms of persistent firing in TRN neurons

My results extend recent work that has implicated TRPM4 in controlling neuronal excitability in several other brain areas. Intracellular recordings from cortical neurons have shown that after pharmacologically blocking TRMP4, an afterdepolarization potential is reduced (Lei et al., 2014; Riquelme et al., 2018). Additionally, pharmacologically blocking TRPM4 in substantia nigra or brainstem neurons resulted in a loss of a plateau potentials (Mrejeru et al., 2011; Picardo et al., 2019). Furthermore, TRPM4 knockout mice provide evidence that TRPM4 activation enhances NMDA-dependent LTP in the hippocampus by depolarizing the membrane and facilitating NMDA activation (Menigoz et al., 2016). Other studies have found that in TRPM4 KO animals a depolarization induced slow current is reduced but not completely eliminated (Kim et al., 2013).. My findings in the TRN are an important extension of these studies and show the necessity of TRPM4 in generating PF.

It should be stated that the majority of TRP antagonists employed in previous studies have a number of non- specific effects. For example, 9-phenthranol is not as selective as previously assumed and can target TRPM5 (Guinamard et al., 2014; Lei et al., 2014), and, based on my own observations, may target K^+ channels. So far, CBA has been shown to be selective for TRPM4 and not TRPM5 (Ozhatil et al., 2018, Leiva-Salcedo et al., 2019), but my findings will require independent confirmation under similar experimental conditions.

A unique property of TRPM4 activation within physiological ranges is the requirement for both membrane depolarization and large increases in intracellular Ca^{2+} concentration (Launay et al., 2002; Nilius et al., 2003; Nilius et al, 2004). Recent evidence from cryo-electron microscopy studies have revealed a calcium binding site between the S2-S3 linker domain of TRPM4 channels. By imaging TRPM4 in the presence of Ca^{2+}, Autzen and colleagues (2018) found that when calcium is bound to TRPM4, there is a conformational shift in the molecular structure similar to that of a voltage dependent potassium channel. Electrophysiological studies have revealed that TRPM4 has voltage dependent properties (Launay et al., 2002; Nilius et al., 2003). Using excised patch techniques and applying large depolarizing current steps outside the range of normal physiological activity TRPM4 channels can be activated in a voltage-dependent manner even in the absence of calcium. However with calcium added to the bath solution, the voltage required to activate TRPM4 was greatly reduced suggesting that the presence of calcium lowers the voltage threshold needed to activate TRPM4 to a physiological range. Taken together TRPM4 channels require a calcium source to allow for voltage dependent activation in physiological ranges.

What are the Ca^{2+} sources of TRPM4 activation? I found that pharmacological block of T-type Ca^{2+} channel using the specific antagonist TTA-P2 completely eliminated PF. Furthermore, by

holding the membrane potential at -60 mV, thereby inactivation t-type Ca2+ channels, I found that PF was absent. Additionally, replacing Ca^{2+} with Ba^{2+}, which T-type Ca2+ channels are permeable to, abolished PF. These findings suggest that the Ca^{2+} increases required for TRPM4 activation are mediated by influx through T-type Ca^{2+} channels although I cannot rule out a contribution of R-type Ca^{2+} channels. R-type Ca^{2+} channels have been linked to T-type activation (Zaman et al., 2011), so it is possible that R-type Ca^{2+} channels facilitate the activation of TRPM4. Furthermore, I cannot rule out a contribution of Ca^{2+}-dependent Ca^{2+} release from internal stores (Neyer et al., 2016) which can activate other Ca^{2+} dependent mechanisms near T-type Ca^{2+} channels.

Like other Ca^{2+} channels, T-type channels can be tightly associated with calcium-dependent mechanisms allowing for local calcium activation (Fakler & Adelman, 2008; Turner & Zamponi, 2014). Moreover, the currents generated by T-type calcium channels results in a large membrane depolarization (Huguenard & Prince, 1992) giving T-type calcium channels the ability to control both calcium dynamics and membrane potentials. Given the calcium- and voltage-dependence of TRPM4, and the dependence on T-type calcium channels, it is highly probable that T-type calcium and TRPM4 are closely associated and may even form microdomains. TRPM4 can closely associate with other channels and even heteromerize with TRPC3 and SUR1 as well as others (Park et al., 2008; Woo et al., 2013), providing further credence to TRPM4 – T-type calcium channel microdomains. Where do TRPM4 and T-type Ca^{2+}channels co-localize? Cav3.3 channels mediating I_T are primarily expressed in the distal dendrites of TRN neurons and their activation during burst firing results in large dendritic but more modest somatic Ca^{2+} signals (Crandall et al., 2010; Astori et al., 2011). This would implicate a dendritic location of TRPM4. In this scenario, membrane depolarizations necessary for TRPM4 activation would likely be mediated by synaptically-generated T-type dependent Ca^{2+} spikes, which

in contrast to fast Na^+ action potentials, propagate effectively into TRN dendrites (Crandall et al., 2010; Connelly et al., 2015).

To provide evidence for the presence of TRPM4 – T-type micrdomains could be accomplished by showing the differential effect of BAPTA and EGTA in the internal solution. However, with the rundown of PF, these experiments are not possible. Therefore, future studies should use immunohistochemical techniques to examine if T-type channels and TRPM4 are closely associated and determine the location of TRPM4 within TRN cells. This will provide a better understanding of how PF can be triggered by T-type calcium channels and how the selectivity of inputs may drive TRPM4 activation.

During whole-cell recordings of TRN cells I observed a rundown of PF. Given that this rundown was not observed during network activity using loose-seal and VB recordings, the rundown is likely due to one or several cell-intrinsic mechanisms. Given that T-type Ca^{2+} channels are necessary for PF, one possible explanation could be the rundown of calcium signals from T-type channels in the distal dendrites lead to the loss of PF over time. Alternatively, recordings in whole-cell mode might have led to the dialysis of critical signaling molecules. TRPM4 conductances rapidly desensitize in excised patches (Zhang et al., 2005) and in whole-cell mode, but modulators such as PIP2 can restore TRPM4 activity. Additional investigations into the mechanisms of TRPM4 regulation under physiological conditions will be important.

4.2 Plateau potentials in the brain – properties and functions

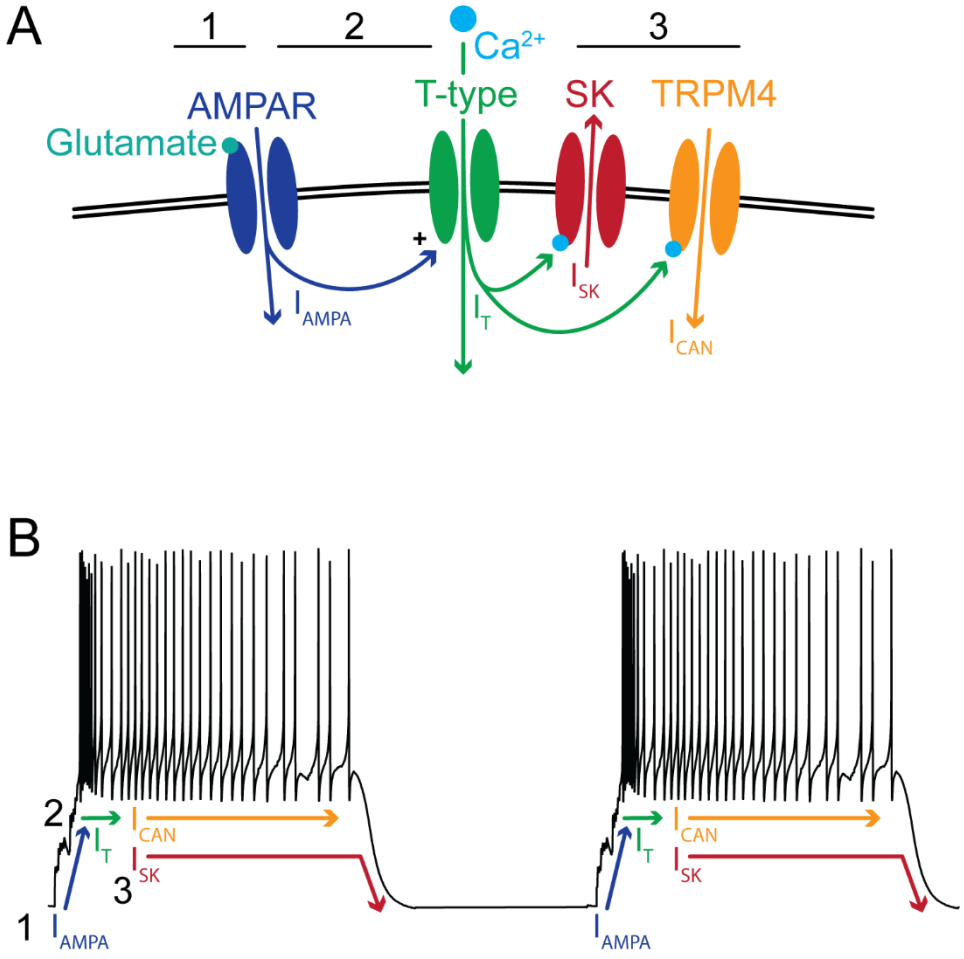
Plateau potentials and PF lasting hundreds of milliseconds to tens of seconds have been observed throughout the brain. PF it has been implicated in a variety of functions such as working

memory (Zylberberg & Strowbridge, 2017), modulation of excitability (Mrejeru et al., 2011) and control of rhythmic activity (Picardo et al., 2019). Much of what is known comes from studies on PF observed in cortex (Major & Tank, 2004; Zylberberg & Strowbridge, 2017), but similar types of activity patterns have been found in subcortical areas (Pressler & Regehr, 2013). In the cortex, PF is thought to maintain activity necessary for working memory and occurs through the interplay of the cholinergic system with cell-intrinsic mechanisms. Application of carbachol results in PF and pharmacological antagonists show the involvement of L-type calcium channels in the entorhinal and anterior cingulate cortices (Egorov et al, 2002; Zhang & Séguéla, 2010), an ether-a-go-go (ERG) current in the PFC (Cui & Strowbridge, 2018), and TRPC channels in the entorhinal cortex (Zhang et al., 2011). Taken together, these findings implicate that PF is functionally relevant throughout the brain and relies on several mechanisms; however, this list is not exhaustive and several other mechanisms have been suggested and yet to be identified.

During sleep and anesthesia the thalamus generates slow oscillations, with activity often characterized by PF mediated by plateau potentials. Several mechanisms have been implicated in the generation and mediation of these plateaus. Kim and McCormick (Kim & Mccormick, 1998) reported plateau potentials in a fraction of neurons of the perigeniculate sector of the ferret TRN, with very similar properties as described here. However, they concluded that plateau potentials were mediated by I_{NaP}, since they were fully blocked by TTX. The same conclusion was reached in an *in vivo* study of TRN in anesthetized cats, which demonstrated block of persistent firing using intracellular dialysis of a voltage-dependent Na^+ channel antagonist (Fuentealba et al., 2005). Crunelli and colleagues have shown that show PF and plateau potentials in the thalamus are generated in an mGluR-dependent manner. For both TRN and thalamic relay neurons, group I mGluR activation triggered rhythmic forms of PF, mediated by the reduction of K^+ leak conductances, allowing the generation of a long-

lasting inward current mediated by the non-inactivating portion of T-type Ca^{2+} channel conductance (Hughes et al., 2002; Blethyn et al., 2006). Furthermore, their computational model of mGluR mediated PF in the TRN showed that PF could be generated in the absence of an I_{CAN}. My findings differ from these results in several important aspects (Fig. 4.1): First, PF did not require mGluR activation and the resulting closure of K^+ leak conductances. Second, in the absence of mGluR activation, PF did not occur in a cell-intrinsic manner but required fast synaptic inputs. Under my experimental conditions, both cholinergic inputs and bursts of glutamatergic thalamic inputs triggered PF, likely by generating global Ca^{2+} spikes (Connelly et al., 2015). Third, while T-type Ca^{2+} channels were important for the initiation of PF, my results are inconsistent with a critical role of I_T in generating long-lasting 'window' currents that lead to plateau potentials. I found that plateau potentials reached levels of ∼-45 mV, where T-type Ca^{2+} conductances at steady-state experience near full inactivation. Furthermore, the lack of PF in the presence of Ba^{2+}, which should allow for the generation of a I_T-mediated window current (Huguenard & Prince, 1992) suggests that T-type Ca^{2+} currents alone are unlikely to mediate PF. Fourth, using the potent and selective antagonist CBA (Ozhatil et al., 2018), I showed that I_{CAN} was essential for the generation of plateau potentials and was mediated by TRPM4. Lastly the underlying plateau potential was insensitive to TTX, implicating that I_{NAP} is not necessary. However, that does not rule out their possible facilitatory role to TRPM4 conductances. For example, in the presence of TTX I found a significant reduction in the proportion of cells that generated a plateau potential. This is likely due to the reduction in overall excitability of from the lack of Na+ currents.

Figure 4.1. Working model of the mechanisms that generate rhythmic PF in the TRN. A. shows the mechanisms that I have found to be involved in generating rhythmic activity and PF in the TRN. **B.** Under my experimental conditions, spontaneous activity from the thalamus generates AMPAR-mediated (*blue*) membrane depolarizations (1). The depolarization from AMPA currents activates T-type calcium channels (*green*), further depolarizing the cell and generating an influx of calcium (2). Calcium can then activate TRPM4 (*yellow*) and SK (*red*) channels resulting in PF and the eventual return to a hyperpolarized membrane (3).


A
1
2
3
Ca2+
AMPAR
Glutamate
T-type
SK
TRPM4
+
I AMPA
I T
I SK
I CAN
B
2
I T
I CAN
I SK
3
1
I AMPA
I AMPA
I T
I CAN
I SK

These differences could be due to differences in experimental design, as they relied of mGluR activation by means of pharmacological activation. However, under physiological conditions, mGluR activation in the TRN requires robust and sustained activity, and in vivo recordings show that activity of corticothalamic cells is reduced during SWS Steriade et al., 2001). This suggests that their experimental conditions result in an artificial oscillatory activity and PF.

4.3 Modulation of PF and implications for *in vivo* conditions.

Several studies have found distinct electrophysiological, characteristics indicative of TRN cell types (Lee, Govindaiah, & Cox, 2007; Clemente-Perez et al., 2017).The evidence from my experiments leads me to conclude that PF is not a property of a distinct cell type. Two key pieces of evidence support this claim. First, I observed PF in virtually all cells when SK channels are blocked, suggesting that the mechanisms are expressed in all TRN cells. Second, PF could be modulated in a given cell, either via intrinsic mechanisms or following activation of synaptic inputs. Taken together, PF is likely undergoing modulatory control from several cell-intrinsic and synaptic mechanisms that actively enhance or suppress PF.

SK is likely able to modulate PF due to its close association with T-type Ca^{2+} channels which results in reliable and strong hyperpolarization of the membrane potential (Cueni et al., 2008; Turner & Zamponi, 2014). Genetic ablation and pharmacological antagonisms of SK channels results in increased excitability observed during epileptiform activity (Cueni et al., 2008; Kleiman-Weiner et al., 2009). Mouse models of autism (PTCHD1; Wells et al., 2016) and Dravet Syndrome (Scn1a; Ritter-Makinson et al., 2019) show increased excitability during intracellular stimulation, due to a loss of SK function in TRN neurons. Together these studies highlight the regulatory role of SK in reducing TRN excitability. The fact that PF could be observed in most recordings in the presence of apamin suggests that SK channels control both the initiation and duration of PF, likely due to the

close functional association with T-type Ca^{2+} channels (Cueni et al., 2008). Given that TRPM4 may also closely associated with T-type channels, SK conductances could actively oppose TRPM4 activation resulting in a "push-pull" between these two conductances allowing for SK to modulate PF.

Similarly, I found that the synaptic activation of M2 muscarinic receptors curtailed the duration of PF. This is most likely due to the hyperpolarizing currents generated by the GIRK channels coupled to mAChRs (Sun et al., 2013). Additionally, mAChRs may directly attenuate T-type channels reducing T-type mediated conductances (Pita-Almenar et al., 2014a). Since the generation of PF is dependent on T-type calcium channel conductances, these findings suggest there are multiple means by which mAChRs can suppress PF. Given that activity of the cholinergic system is elevated during arousal (Diekelmann & Born, 2010; Adamantidis et al., 2019), it is likely that mAChR actively work to reduce PF, without fully blocking TRN neuronal activation.

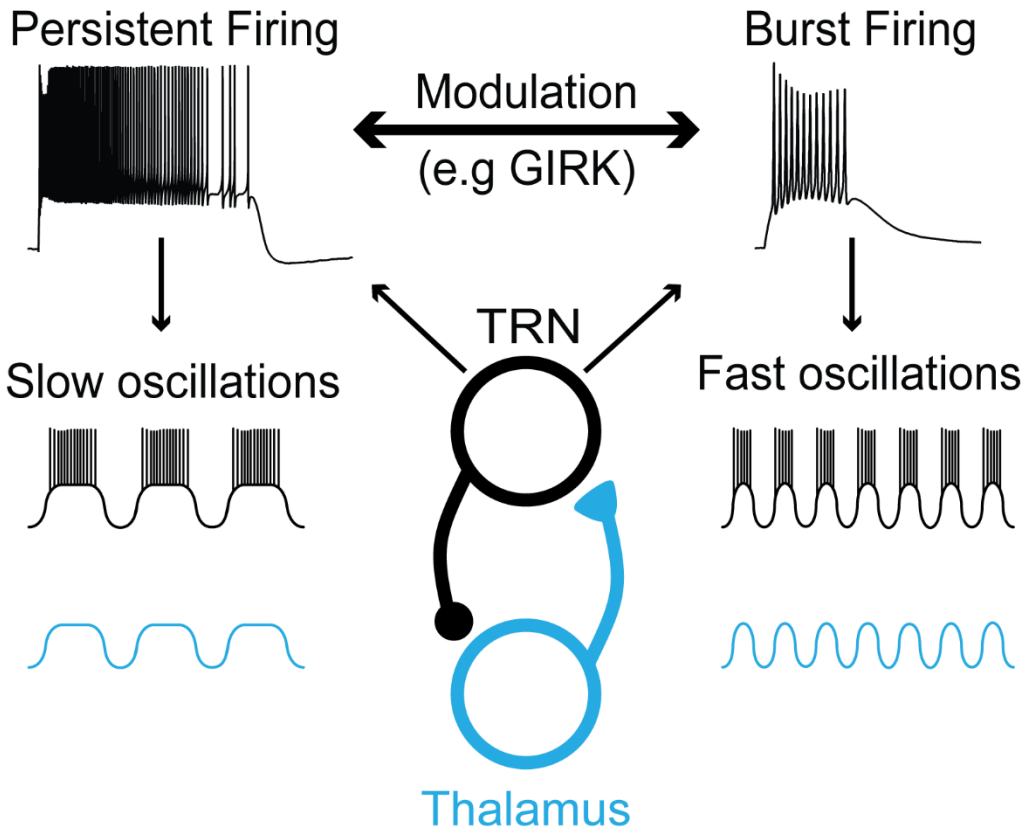
The dynamic modulation of PF, coupled with the low incidence of PF and the small number of *in vivo* TRN studies may explain why PF has not been reported *in vivo*. It is therefore likely that under physiological conditions, TRPM4 activation and the generation of PF in a given neuron does not occur in all-or-none manner, but instead is constantly regulated by multiple synaptic and intrinsic processes. For example, an increased likelihood and duration of PF might be promoted by mechanisms that increase T-type mediated Ca^{2+} increases or directly facilitate TRPM4 activation such as PIP2 (Zhang et al., 2005), and curtailed by modulatory synaptic systems that reduce TRN excitability via opening of specific K conductances. Thus there is a need for further investigation into the mechanisms that modulate TRPM4 conductances with particular emphasis on *in vivo* studies.

4.4 Function of PF in the TRN

Prolonged bursts from the TRN, lasting 10s of ms, can activate $GABA_B$ (Kim et al., 1997) and extrasynaptic $GABA_A$ receptors. At many synapses including GABAergic inputs to thalamic neurons, extrasynaptic receptors are usually not recruited by GABA release following a single action potential, due to rapid transmitter clearance. However following bursts or PF and sustained release of GABA, saturation of uptake results in "spill-over" of GABA and the recruitment of extrasynaptic receptors. Given the inverse relationship between TRN firing duration and oscillatory frequency I observed, it is tempting to suggest that PF acts to reduce the frequency of thalamic oscillations, via recruitment of $GABA_B$ receptors (Kim et al., 1997) or of extrasynaptic high-affinity $GABA_A$ receptors (Herd et al., 2013), thereby changing the latency to rebound burst generation (Schofield et al., 2009). Furthermore, the frequency of oscillatory activity in the TRN may lead to long-term plasticity of TRN inputs onto thalamic cells (Sieber et al., 2013; Pigeat et al., 2015).

Beyond the TRN, TRPM4 mediated PF has been implicated in shaping the frequency of oscillatory activity. Interneurons in the pre-Bötzinger complex of the brainstem express TRPM4 and shape rhythmic activity necessary for breathing rhythms (Picardo et al., 2019). By genetically knocking down TRPM4, or pharmacologically blocking TRPM4 during rhythmic activity, it was found that the interneurons could still generate rhythmic activity, but the frequency of breathing rhythms was reduced, suggesting that TRPM4 is involved in shaping oscillatory frequencies. Given that $GABA_B$ can modulate TRN oscillatory activity (Jacobsen, et al., 2001) it is likely that PF slows oscillatory frequency by activating $GABA_B$ receptors. Moreover, the fact that TRPM4 mediated PF is under dynamic and modulatory control by various mechanisms is likely that these mechanisms work in tandem to shape the oscillations by modulating PF (Figure 4.2).

Figure 4.2. Dynamic modulation of PF shapes intrathalamic oscillations. A working model depicting how the TRN can dynamically control the intrathalamic slow oscillations. TRN neurons shift their firing properties between short bursts of action potentials (Burst Firing) and prolonged activity (Persistent Firing). It is very likely that this activity exists on a continuum and channels such as GIRK, SK, or other unidentified mechanisms work to reduce the duration of TRN firing. The duration of TRN firing may control intrathalamic oscillation frequency through $GABA_B$ receptor activation. Modulation by GIRK or SK acts as a regulator of PF and may work to increase the frequency of intrathalamic oscillations.


Persistent Firing
Modulation
(e.g GIRK)
Burst Firing
TRN
Slow oscillations
Fast oscillations
Thalamus

Alternatively, sustained GABA release from TRN PF may activate presynaptic $GABA_B$ receptors. Pharmacological activation of presynaptic $GABA_B$ receptors on lemniscal inputs resulted in a reduction in firing from these inputs (Emri et al., 1996) suggesting that PF may aid in the sensory disconnect during slow wave sleep. Furthermore, there are presynaptic $GABA_B$ receptors on TRN inputs into thalamus, and pharmacological antagonism of $GABA_B$ receptors resulted in increased IPSCs in the VB (Le Feuvre et al., 1997). Activation of these receptors has been implicated in the oscillatory activity suggesting that PF may alter the frequency by activation of these presynaptic receptors. Taken together, it is very likely that the functional role of PF in the TRN results in enhanced release of GABA and the activation of both pre- and postsynaptic $GABA_A$ and $GABA_B$ receptors.

4.5 PF and network driven slow oscillations

Accumulating evidence indicates that both cortex and thalamus can generate oscillations in isolation, suggesting that rhythmic activity in the intact thalamocortical system results from the complex interplay of multiple distinct oscillators (Crunelli & Hughes, 2010). While there has been extensive research on the cortical mechanisms mediating slow rhythms, the nature of thalamic pacemakers are less well understood. Previous work has shown that both TRN and relay neurons are capable of generating cell-intrinsic rhythms in the 1 Hz range, but only under conditions of postsynaptic mGluR activation (Hughes et al., 2002; Blethyn et al., 2006). Under physiological conditions, this would require sustained corticothalamic activity, indicating that both thalamic cell types act as conditional oscillators. My findings derived from adult thalamic slices highlight an alternative mechanism and suggest that networks of TRN and VB cells can generate robust and long-lasting slow oscillatory activity, mediated by powerful bidirectional synaptic connectivity (Gentet & Ulrich, 2003; Pinault, 2004; Pita-Almenar et al., 2014b).

My network data suggest that robust thalamic rhythmicity can occur in the absence of precise synchrony, highlighting that both processes can occur independently. While thalamic oscillatory activity is commonly referred to as synchronous (Fogerson & Huguenard, 2016), there have been few studies that have directly examined the degree of precise thalamic synchronization during distinct behavioral states. My current data showing a lack of precise synchrony in local thalamic circuits confirm previous findings (Pita-Almenar et al., 2014b; Fogerson & Huguenard, 2016) and indicate that the variability in TRN firing might act as an additional factor in preventing synchrony in isolated thalamic networks. Furthermore, other than electrical synapses interconnecting local TRN neurons (Landisman et al., 2002) there is no mechanism in place in the intrathalamic network that can generate synchrony. The presence of synchrony in in vivo recordings therefore suggest that it is generated by external drive. It has been suggested that synchronization of thalamic networks is primarily controlled by cortical feedback connections (Contreras & Steriade, 1996). However, these recordings were performed under anesthesia which locks the thalamocortical system into a highly synchronous state. During natural sleep, activity in cortex shows less synchrony and activity in TRN and thalamus is not reliably entrained by cortex (Urbain et al., 2019).

4.6 Concluding remarks

In summary, I have shown that the slow oscillations in the TRN are generated by network activity resulting from the reciprocal connections between the TRN and thalamus, and that TRPM4 shapes the TRN firing pattern and may modulate rhythmicity. Specifically, my work identified TRPM4 as the molecular mechanism of I_{CAN} and showed the necessity of I_{CAN} in generating the plateau potentials during rhythmic activity. Dysfunction of the TRN are present in various disease models with disruptions to attention and/or sleep. These disruptions are also seen in human patients

and underscore the critical importance of the TRN in disorders like Alzheimer's disease, autism, or schizophrenia. Therefore, a better understanding of the molecular and cellular mechanisms of TRN (dys)function might provide important therapeutic avenues to a number of devastating neurological diseases.

References

Adamantidis, A. R., Gutierrez Herrera, C., & Gent, T. C. (2019). Oscillating circuitries in the sleeping brain. *Nature Reviews Neuroscience*, *20*(12), 746–762. https://doi.org/10.1038/s41583-019-0223-4

Agmon, A., & Connors, B. W. (1991). Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience*, *41*(2–3), 365–379. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1870696

Alexander, G. M., & Godwin, D. W. (2006). Unique presynaptic and postsynaptic roles of group II metabotropic glutamate receptors in the modulation of thalamic network activity. *Neuroscience*, *141*(1), 501–513. https://doi.org/10.1016/j.neuroscience.2006.03.060

Amzica, F., & Steriade, M. (1995). Short- and long-range neuronal synchronization of the slow (<1 Hz) cortical oscillation. *Journal of Neurophysiology*, *73*(1), 20–38. https://doi.org/10.1152/jn.1995.73.1.20

Asanuma, C. (1992). Noradrenergic innervation of the thalamic reticular nucleus: A light and electron microscopic immunohistochemical study in rats. *The Journal of Comparative Neurology*, *319*(2), 299–311.

Asanuma, C. (1994). GABAergic and pallidal terminals in the thalamic reticular nucleus of squirrel monkeys. *Experimental Brain Research*, *101*(3), 439–451. https://doi.org/10.1007/BF00227337

Astori, S., Wimmer, R. D., Prosser, H. M., Corti, C., Corsi, M., Liaudet, N., Volterra, A., Franken, P. Adelman, J. P., & Lüthi, A. (2011). The Ca v3.3 calcium channel is the major sleep spindle pacemaker in thalamus. *Proceedings of the National Academy of Sciences of the United States*

of America, *108*(33), 13823–13828. https://doi.org/10.1073/pnas.1105115108

Autzen, H. E., Myasnikov, A. G., Campbell, M. G., Asarnow, D., Julius, D., & Cheng, Y. (2018). Structure of the human TRPM4 ion channel in a lipid nanodisc. *Science*, *359*(6372), 228–232. https://doi.org/10.1126/science.aar4510

Bal, B. Y. T., & Mccormick, D. A. (1993). Mechanisms of oscillatory activity in guinea-pig nucleus reticularis thalami in vitro: A mammalian pacemaker. *Journal of Neurophysiology*, *468*, 669–691.

Bazhenov, M., Timofeev, I., Steriade, M., & Sejnowski, T. J. (1999). Self-sustained rhythmic activity in the thalamic reticular nucleus mediated by depolarizing GABAA receptor potentials. *Nature Neuroscience, 2*, 168-174.

Beenhakker, M. P., & Huguenard, J. R. (2009). Neurons that Fire Together Also Conspire Together: Is Normal Sleep Circuitry Hijacked to Generate Epilepsy? *Neuron*, *62*(5), 612–632. https://doi.org/10.1016/j.neuron.2009.05.015

Beierlein, M. (2014). Synaptic mechanisms underlying cholinergic control of thalamic reticular nucleus neurons. *Journal of Physiology*, *592*(19), 4137–4145. https://doi.org/10.1113/jphysiol.2014.277376

Ben-Mabrouk, F., & Tryba, A. K. (2010). Substance P modulation of TRPC3/7 channels improves respiratory rhythm regularity and ICAN-dependent pacemaker activity. *European Journal of Neuroscience*, *31*(7), 1219–1232. https://doi.org/10.1111/j.1460-9568.2010.07156.x

Bickford, M. E., Günlük, A. E., van Horn, S. C., & Sherman, S. M. (1994). GABAergic projection from the basal forebrain to the visual sector of the thalamic reticular nucleus in the cat. *Journal of Comparative Neurology*, *348*(4), 481–510. https://doi.org/10.1002/cne.903480402

Blethyn, K. L., Hughes, S. W., Tóth, T. I., Cope, D. W., & Crunelli, V. (2006). Neuronal basis of the slow (<1 Hz) oscillation in neurons of the nucleus reticularis thalami in vitro. *Journal of Neuroscience*, *26*(9), 2474–2486. https://doi.org/10.1523/JNEUROSCI.3607-05.2006

Brill, J., Kwakye, G., & Huguenard, J. R. (2007). NPY signaling through Y1 receptors modulates thalamic oscillations. *Peptides*, *28*(2), 250–256. https://doi.org/10.1016/j.peptides.2006.08.043

Bryant, A. S., Li, B., Beenhakker, M. P., & Huguenard, J. R. (2009). Maintenance of thalamic epileptiform activity depends on the astrocytic glutamate-glutamine cycle. *Journal of Neurophysiology*, *102*(5), 2880–2888. https://doi.org/10.1152/jn.00476.2009

Bucherelli, C., Tassoni, G., & Bures, J. (1993). Differential effect of functional ablation of thalamic reticular nucleus on the acquisition of passive and active avoidance. *International Journal of Neuroscience*, *73*(1–2), 77–84. https://doi.org/10.3109/00207459308987213

Burgunder, J. M., & Young III, W. S. (1990). Cortical neurons expressing the cholecystokinin gene in the rat: Distribution in the adult brain, ontogeny, and some of their projections. *The Journal of Comparative Neurology*, *300*(1), 26–46.

Chang, S. Y., Zagha, E., Kwon, E. S., Ozaita, A., Bobik, M., Martone, M. E., Ellisman, M. H.

Heintz, N., & Rudy, B. (2007). Distribution of Kv3.3 potassium channel subunits in distinct neuronal populations of mouse brain. *The Journal of Comparative Neurology*, *502*(6), 953–972.

Chklovskii, D. B. (2004). Synaptic connectivity and neuronal morphology: Two sides of the same coin. *Neuron*, *43*(5), 609–617. https://doi.org/10.1016/j.neuron.2004.08.012

Clemente-Perez, A., Makinson, S. R., Higashikubo, B., Brovarney, S., Cho, F. S., Urry, A., Holden, S. S., Wimer, M., Dávid, C., Fenno, L. E., Acsády, L., Deisseroth, K., & Paz, J. T. (2017).

Distinct Thalamic Reticular Cell Types Differentially Modulate Normal and Pathological Cortical Rhythms. *Cell Reports*, *19*(10), 2130–2142. https://doi.org/10.1016/j.celrep.2017.05.044

Collery, M., M'Harzi, M., & Delacour, J. (1993). Lesions of reticularis thalamic nucleus impair spatial working memory in rats. *Neuroscience Research Communications*, *12*(1), 41–49.

Connelly, W. M., Crunelli, V., & Errington, A. C. (2015). The global spike: Conserved dendritic properties enable unique ca2+ spike generation in low-threshold spiking neurons. *Journal of Neuroscience*, *35*(47), 15505–15522. https://doi.org/10.1523/JNEUROSCI.2740-15.2015

Connors, B. W., & Long, M. A. (2004). Electrical Synapses in the Mammalian Brain. *Annual Review of Neuroscience*, *27*(1), 393–418. https://doi.org/10.1146/annurev.neuro.26.041002.131128

Contreras, D., & Steriade, M. (1996). Synchronization of low-frequency rhythms in corticothalamic networks. *Neuroscience*, *76*(1), 11–24. https://doi.org/10.1016/S0306-4522(96)00393-4

Coulon, P., & Landisman, C. E. (2017). The potential role of gap junctional plasticity in the regulation of state. *Neuron, 93*(6) 1275-1295.

Cox, C. L., Huguenard, J. R., & Prince, D. A. (1995). Cholecystokinin depolarizes rat thalamic reticular neurons by suppressing a K+ conductance. *Journal of Neurophysiology*, *74*(3), 990–1000. https://doi.org/10.1152/jn.1995.74.3.990

Cox, Charles L., & Sherman, S. M. (1999). Glutamate inhibits thalamic reticular neurons. *Journal of Neuroscience*, *19*(15), 6694–6699. https://doi.org/10.1523/jneurosci.19-15-06694.1999

Crabtree, J. W. (1996). Organization in the somatosensory sector of the cat's thalamic reticular nucleus. *Journal of Comparative Neurology*, *390*(2), 167–182.

https://doi.org/10.1002/(sici)1096-9861(19980112)390:2<167::aid-cne1>3.0.co;2-%23

Crabtree, J. W. (1998). Organization in the auditory sector of the cat's thalamic reticular nucleus. *The Journal of Comparative Neurology*, *390*, 167–182.

Crabtree, J. W. (2018). Functional diversity of thalamic reticular subnetworks. *Frontiers in Systems Neuroscience*, *12*(October), 1–18. https://doi.org/10.3389/fnsys.2018.00041

Crabtree, J. W., Collingridge, G. L, & Isaac, J. T. R. (1998). A new intrathalamic pathway linking modality-related nuclei in the dorsal thalamus. *Nature Neuroscience, 1*, 389-394.

Crabtree, J. W., & Isaac, J.T. R. (2002) New intrathalamic pathways allowing modality-related and cross-modality switching in the dorsal thalamus. *Journal of Neuroscience, 22*(19), 8754-8761.

Crabtree, J. W., Lodge, D., Bashir, Z. I., & Isaac, J. T. R. (2013). GABAA, NMDA and mGlu2 receptors tonically regulate inhibition and excitation in the thalamic reticular nucleus. *European Journal of Neuroscience*, *37*(6), 850–859. https://doi.org/10.1111/ejn.12098

Crandall, S. R., Cruikshank, S. J., & Connors, B. W. (2015). A Corticothalamic Switch: Controlling the Thalamus with Dynamic Synapses. *Neuron*, *86*(3), 768–782. https://doi.org/10.1016/j.neuron.2015.03.040

Crandall, S. R., Govindaiah, G., & Cox, C. L. (2010). Low-threshold Ca 2+ current amplifies distal dendritic signaling in thalamic reticular neurons. *Journal of Neuroscience*, *30*(46), 15419–15429. https://doi.org/10.1523/JNEUROSCI.3636-10.2010

Crick, F. (1984). Function of the thalamic reticular complex: The searchlight hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *81*(14 I), 4586–4590. https://doi.org/10.1073/pnas.81.14.4586

Cropper, E. C., Eisenman, J. S., & Azmitia, E. C. (1984). An immunocytochemical study of the

serotonergic innervation of the thalamus of the rat. *The Journal of Comparative Neurology*, *224*(1), 38–50.

Cruikshank, S. J., Rose, H. J., & Metherate, R. (2002). Auditory thalamocortical synaptic transmission in vitro. *Journal of Neurophysiology*, *87*(1), 361–384. https://doi.org/10.1152/jn.00549.2001

Crunelli, V., & Hughes, S. W. (2010). The slow (1 Hz) rhythm of non-REM sleep: A dialogue between three cardinal oscillators. *Nature Neuroscience*, *13*(1), 9–17. https://doi.org/10.1038/nn.2445

Crunelli, V., Larincz, M. L., Connelly, W. M., David, F., Hughes, S. W., Lambert, R. C., Leresche, N., & Errington, A. C. (2018). Dual function of thalamic low-vigilance state oscillations: Rhythm-regulation and plasticity. *Nature Reviews Neuroscience*, *19*(2), 107–118. https://doi.org/10.1038/nrn.2017.151

Crunelli, V., Lorincz, M. L., Errington, A. C., & Hughes, S. W. (2012). Activity of cortical and thalamic neurons during the slow (<1 Hz) rhythm in the mouse in vivo. *Pflugers Archiv European Journal of Physiology*, *463*(1), 73–88. https://doi.org/10.1007/s00424-011-1011-9

Csillik, B., Mihály, A., Krisztin-Péva, B., Chadaide, Z., Samsam, M., Knyihár-Csillik, E., & Fenyo, R. (2005). GABAergic parvalbumin-immunoreactive large calyciform presynaptic complexes in the reticular nucleus of the rat thalamus. *Journal of Chemical Neuroanatomy*, *30*(1), 17–26. https://doi.org/10.1016/j.jchemneu.2005.03.010

Cueni, L., Canepari, M., Luján, R., Emmenegger, Y., Watanabe, M., Bond, C. T., Franken, P., Adelman, J. P., & Lüthi, A. (2008). T-type Ca2+ channels, SK2 channels and SERCAs gate sleep-related oscillations in thalamic dendrites. *Nature Neuroscience*, *11*(6), 683–692.

https://doi.org/10.1038/nn.2124

Cui, E. D., & Strowbridge, B. W. (2018). Modulation of ether-à-Go-Go related gene (ERG) current governs intrinsic persistent activity in rodent neocortical pyramidal cells. *Journal of Neuroscience*, *38*(2), 423–440. https://doi.org/10.1523/JNEUROSCI.1774-17.2017

Deleuze, C. & Huguenard, J. R. (2006). Distinct electrical and chemical connectivity maps in the thalamic reticular nucleus: Potential roles in synchronization and sensation. *Journal of Neuroscience, 26*(33), 8633-8645.

Destexhe, A., Contreras, D., Sejnowski, T. J., & Steriade, M. (1994). Modeling the control ofreticular thalamic oscillations by neuromodulators. *NeuroReports*, *5*, 2217–2220.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience*, *11*(2), 114–126. https://doi.org/10.1038/nrn2762

Dong, P., Wang, H., Shen, X. F., Jiang, P., Zhu, X. T., Li, Y., Gao, J. H., Lin, S., Huang, Y., He, X. B., Xu, F. Q., Duan, S., Lian, H., Wang, H., Chen, J., Li, X. M. (2019). A novel cortico-intrathalamic circuit for flight behavior. *Nature Neuroscience*, *22*(6), 941–949. https://doi.org/10.1038/s41593-019-0391-6

Egorov, A. V., Hamam, B. N., Fransén, E., Hasselmo, M. E., & Alonso, A. A. (2002). Graded persistent activity in entorhinal cortex neurons. *Nature*, *420*(6912), 173–178. https://doi.org/10.1038/nature01171

Emri, Z., Turner, J. P., & Crunelli, V. (1996). Tonic activation of presynaptic GABAB Receptors on the thalamic sensory afferents. *Neuroscience*, *72*(3), 689–698.

Espinosa, F., Torres-Vega, M. A., Marks, G. A., & Joho, R. H. (2008). Ablation of Kv3.1 and Kv3.3 potassium channels disrupts thalamocortical oscillations in vitro and in vivo. *Journal of*

Neuroscience, *28*(21), 5570–5581. https://doi.org/10.1523/JNEUROSCI.0747-08.2008

Fakler, B., & Adelman, J. P. (2008). Control of KCa Channels by Calcium Nano/Microdomains. *Neuron*, *59*(6), 873–881. https://doi.org/10.1016/j.neuron.2008.09.001

Fitzgibbon, T., Tevah, L. V., & Sefton, A. J. (1995). Connections between the reticular nucleus of the thalamus and pulvinar-lateralis posterior complex: A WGA-HRP study. *The Journal of Comparative Neurology*, *363*(3), 489–504.

Fogerson, P. M., & Huguenard, J. R. (2016). Tapping the Brakes: Cellular and Synaptic Mechanisms that Regulate Thalamic Oscillations. *Neuron*, *92*(4), 687–704. https://doi.org/10.1016/j.neuron.2016.10.024

Fuentealba, P., Timofeev, I., Bazhenov, M., Sejnowski, T. J., & Steriade, M. (2005). Membrane bistability in thalamic reticular neurons during spindle oscillations. *Journal of Neurophysiology*, *93*(1), 294–304. https://doi.org/10.1152/jn.00552.2004

Fuentealba, P., & Steriade, M. (2005). The reticular nucleus revisited: Intrinsic and network properties of a thalamic pacemaker. *Progress in Neurobiology*, *75*(2), 125–141. https://doi.org/10.1152/jn.00552.2004

Gentet, L. J., & Ulrich, D. (2003). Strong, reliable and precise synaptic connections between thalamic relay cells and neurones of the nucleus reticularis in juvenile rats. *Journal of Physiology*, *546*(3), 801–811. https://doi.org/10.1113/jphysiol.2002.032730

Golshani, P., Liu, X.-B., & Jones, E. G. (2001). Differences in quantal amplitude reflect GluR4-subunit number at corticothalamic synapses on two populations of thalamic neurons. *PNAS, 98*(7), 4172-4177.

Guido, W. (2018). Development, form, and function of the mouse visual thalamus. *Journal of*

Neurophysiology, *120*(1), 211–225. https://doi.org/10.1152/jn.00651.2017

Guillery, R. W. (1995). Anatomical evidence concerning the role of the thalamus in corticocortical communication: a brief review. *Journal of Anatomy*, *187 (Pt 3*, 583–592. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8586557%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1167461

Guillery, R. W., & Harting, J. K. (2003). Structure and connections of the thalamic reticular nucleus: Advancing views over half a century. *Journal of Comparative Neurology*, *463*(4), 360–371. https://doi.org/10.1002/cne.10738

Guinamard, R., Hof, T., & Del Negro, C. A. (2014). The TRPM4 channel inhibitor 9-phenanthrol. *British Journal of Pharmacology*, *171*(7), 1600–1613. https://doi.org/10.1111/bph.12582

Haas, J. S., Zavala, B., & Landisman, C. E. (2011). Activity-dependent long-term depression of electrical synapses. *Science*, *334*(6054), 389–393. https://doi.org/10.1126/science.1207502

Halassa, M. M., Chen, Z., Wimmer, R. D., Brunetti, P. M., Zhao, S., Zikopoulos, B., Wang, F., Brown, E. N., & Wilson, M. A. (2014). State-dependent architecture of thalamic reticular subnetworks. *Cell*, *158*(4), 808–821. https://doi.org/10.1016/j.cell.2014.06.025

Halassa, M. M., Siegle, J. H., Ritt, J. T., Ting, J. T., & Moore, C. I. (2011). Selective optical drive of thalamic reticular nucleus generates thalamic bursts & cortical spindles. *Nature Neuroscience*, *14*(9), 1118–1120. https://doi.org/10.1038/nn.2880.Selective

Hallanger, A. E., & Wainer, B. H. (1988). Ultrastructure of ChAT-immunoreactive synaptic terminals in the thalamic reticular nucleus of the rat. *The Journal of Comparative Neurology*, *278*(4), 486–497.

Hartings, J. A., Temereanca, S., & Simons, D. J. (2003). State-dependent processing of sensory

stimuli by thalamic reticular neurons. *Journal of Neuroscience*, *23*(12), 5264–5271. https://doi.org/10.1523/jneurosci.23-12-05264.2003

Hartmann, J., Dragicevic, E., Adelsberger, H., Henning, H. A., Sumser, M., Abramowitz, J., Blum, R., Dietrich, A., Freichel, M., Flockerzi, V., Birnbaumer, L., & Konnerth, A. (2008). TRPC3 Channels Are Required for Synaptic Transmission and Motor Coordination. *Neuron*, *59*(3), 392–398. https://doi.org/10.1016/j.neuron.2008.06.009

Hazra, A., Corbett, B. F., You, J. C., Aschmies, S., Zhao, L., Li, K., Lepore, A. C., Marsh, E. D., & Chin, J. (2016). Corticothalamic network dysfunction and behavioral deficits in a mouse model of Alzheimer's disease. *Neurobiology of Aging*, *44*, 96–107. https://doi.org/10.1016/j.neurobiolaging.2016.04.016

Hefft, S., & Jonas, P. (2005). Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. *Nature Neuroscience*, *8*(10), 1319–1328. https://doi.org/10.1038/nn1542

Herd, M. B., Brown, A. R., Lambert, J. J., & Belelli, D. (2013). Extrasynaptic GABAA receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *Journal of Neuroscience*, *33*(37), 14850–14868. https://doi.org/10.1523/JNEUROSCI.1174-13.2013

Herrera, C. G., Cadavieco, M. C., Jego, S., Ponomarenko, A., Korotkova, T., & Adamantidis, A. (2016). Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. *Nature Neuroscience*, *19*(2), 290–298. https://doi.org/10.1038/nn.4209

Hirata, A., Aguilar, J., & Castro-Alamancos, M. A. (2006). Noradrenergic activation amplifies bottom-up and top-down signal-to-noise ratios in sensory thalamus. *Journal of Neuroscience*, *26*(16), 4426–4436. https://doi.org/10.1523/JNEUROSCI.5298-05.2006

Hormuzdi, S. G., Pais, I., LeBeau, F. E. N., Towers, S. K., Rozov, A., Buhl, E. H., Whittington, M.s A., & Monyer, H. (2001). Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron*, *31*(3), 487–495. https://doi.org/10.1016/S0896-6273(01)00387-7

Hou, G., Smith, A. G., & Zhang, Z. W. (2016). Lack of intrinsic GABAergic connections in the thalamic reticular nucleus of the mouse. *Journal of Neuroscience*, *36*(27), 7246–7252. https://doi.org/10.1523/JNEUROSCI.0607-16.2016

Huang, Q., Zhou, D., Chase, K., Gusella, J. F., Aronin, N., & Difiglia, M. (1992). Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- And postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(24), 11988–11992. https://doi.org/10.1073/pnas.89.24.11988

Huerta-Ocampo, I., Hacioglu-Bay, H., Dautan, D., & Mena-Segovia, J. (2020). Distribution of midbrain cholinergic axons in the thalamus. *ENeuro*, *7*(1), 1–10. https://doi.org/10.1523/ENEURO.0454-19.2019

Hughes, S. W., Cope, D. W., Blethyn, K. L., & Crunelli, V. (2002a). Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. *Neuron*, *33*(6), 947–958. https://doi.org/10.1016/S0896-6273(02)00623-2

Hughes, S. W., Cope, D. W., Tóth, T. I., Williams, S. R., & Crunelli, V. (1999). All thalamocortical neurones possess a T-type Ca2+ “window” current that enables the expression of bistability-mediated activities. *Journal of Physiology*, *517*(3), 805–815. https://doi.org/10.1111/j.1469-7793.1999.0805s.x

Huguenard, J. R., & Prince, D. A. (1992). A novel T-type current underlies prolonged Ca2+-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *Journal of Neuroscience*, *12*(10), 3804–3817. https://doi.org/10.1523/jneurosci.12-10-03804.1992

Huguenard, J. R., & Prince, D. A. (1994a). Clonazepam suppresses GABA(B)-mediated inhibition in thalamic relay neurons through effects in nucleus reticularis. *Journal of Neurophysiology*, *71*(6), 2576–2581. https://doi.org/10.1152/jn.1994.71.6.2576

Huguenard, J. R., & Prince, D. A. (1994b). Intrathalamic rhythmicity studied in vitro: Nominal T-current modulation causes robust antioscillatory effects. *Journal of Neuroscience*, *14*(9), 5485–5502. https://doi.org/10.1523/jneurosci.14-09-05485.1994

Huguenard, John R., & McCormick, D. A. (2007a). Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends in Neurosciences*, *30*(7), 350–356. https://doi.org/10.1016/j.tins.2007.05.007

Huntsman, M. M., Porcello, D. M., Homanics, G. E., DeLorey, T. M., & Huguenard, J. R. (1999). Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. *Science*, *283*(5401), 541–543. https://doi.org/10.1126/science.283.5401.541

Ingram, S. M., Krause, R. G., Baldino, F., Skeen, L. C., & Lewis, M. E. (1989). Neuronal localization of cholecystokinin mRNA in the rat brain by using in situ hybridization histochemistry. *The Journal of Comparative Neurology*, *287*(2), 260–272.

Jacobsen, R. B., Ulrich, D., & Huguenard, J. R. (2001). GABAB and NMDA receptors contribute to spindle-like oscillations in rat thalamus in vitro. *Journal of Neurophysiology*, *86*(3), 1365–1375. https://doi.org/10.1152/jn.2001.86.3.1365

Jiang, X., Shen, S., Cadwell, C. R., Berens, P., Sinz, F., Ecker, A. S., Patel, S., & Tolias, A. S.

(2015). Principles of connectivity among morphologically defined cell types in adult neocortex. *Science*, *350*(6264). https://doi.org/10.1126/science.aac9462

Jurgens, C. W. D., Bell, K. A., McQuiston, A. R., & Guido, W. (2012). Optogenetic Stimulation of the Corticothalamic Pathway Affects Relay Cells and GABAergic Neurons Differently in the Mouse Visual Thalamus. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0045717

Kharazia, V. N., & Weinberg, R. J. (1994). Glutamate in thalamic fibers terminating in layer IV of primary sensory cortex. *Journal of Neuroscience*, *14*(10), 6021–6032. https://doi.org/10.1523/jneurosci.14-10-06021.1994

Kim, A., Latchoumane, C., Lee, S., Kim, G. B., Cheong, E., Augustine, G. J., & Shin, H. S. (2012). Optogenetically induced sleep spindle rhythms alter sleep architectures in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(50), 20673–20678. https://doi.org/10.1073/pnas.1217897109

Kim, U., & Mccormick, D. A. (1998). Functional and ionic properties of a slow afterhyperpolarization in ferret perigeniculate neurons in vitro. *Journal of Neurophysiology*, *80*(3), 1222–1235. https://doi.org/10.1152/jn.1998.80.3.1222

Kim, U., Sanchez-Vives, M. V., & McCormick, D. A. (1997). Functional dynamics of GABAergic inhibition in the thalamus. *Science*, *278*(5335), 130–134. https://doi.org/10.1126/science.278.5335.130

Kim, Y. S., Kang, E., Makino, Y., Park, S., Shin, J. H., Song, H., Launay, P., & Linden, D. J. (2013). Characterizing the conductance underlying depolarization-induced slow current in cerebellar Purkinje cells. *Journal of Neurophysiology*, *109*(4), 1174–1181. https://doi.org/10.1152/jn.01168.2011

Kimura, A., Yokoi, I., Imbe, H., Donishi, T., & Kaneoke, Y. (2012). Distinctions in burst spiking between thalamic reticular nucleus cells projecting to the dorsal lateral geniculate and lateral posterior nuclei in the anesthetized rat. *Neuroscience*, *226*, 208–226. https://doi.org/10.1016/j.neuroscience.2012.09.016

Kimura, Akihisa, & Imbe, H. (2015). Anatomically structured burst spiking of thalamic reticular nucleus cells: Implications for distinct modulations of sensory processing in lemniscal and non-lemniscal thalamocortical loop circuitries. *European Journal of Neuroscience*, *41*(10), 1276–1293. https://doi.org/10.1111/ejn.12874

Kleiman-Weiner, M., Beenhakker, M. P., Segal, W. A., & Huguenard, J. R. (2009). Synergistic roles of GABAA receptors and SK channels in regulating thalamocortical oscillations. *Journal of Neurophysiology*, *102*(1), 203–213. https://doi.org/10.1152/jn.91158.2008

Kohn, A., Zandvakili, A., & Smith, M. A. (2009). Correlations and brain states: from electrophysiology to functional imaging. *Current Opinion in Neurobiology*, *19*(4), 434–438. https://doi.org/10.1016/j.conb.2009.06.007

Kolaj, M., Zhang, L., Hermes, M. L. H. J., & Renaud, L. P. (2014). Intrinsic properties and neuropharmacology of midline paraventricular thalamic nucleus neurons. *Frontiers in Behavioral Neuroscience*, *8*(APR), 1–15. https://doi.org/10.3389/fnbeh.2014.00132

Köliker, A. (1896). *Handbuch der Gewebelehre des Menschen. Nervensystemen des Menschen und der Thiere*. (W. Englemann, Ed.) (6th ed.). Leipzig.

Lam, Y. W., & Sherman, M. S. (2015). Functional topographic organization of the motor reticulothalamic pathway. *Journal of Neurophysiology*, *113*(9), 3090–3097. https://doi.org/10.1152/jn.00847.2014

Lam, Y. W., & Sherman, S. M. (2007). Different topography of the reticulothalamic inputs to first- and higher-order somatosensory thalamic relays revealed using photostimulation. *Journal of Neurophysiology*, *98*(5), 2903–2909. https://doi.org/10.1152/jn.00782.2007

Lam, Y. W., & Sherman, S. M. (2011). Functional organization of the thalamic input to the thalamic reticular nucleus. *Journal of Neuroscience*, *31*(18), 6791–6799. https://doi.org/10.1523/JNEUROSCI.3073-10.2011

Landisman, C. E., & Connors, B. W. (2005). Neuroscience: Long-term modulation of electrical synapses in the mammalian thalamus. *Science*, *310*(5755), 1809–1813. https://doi.org/10.1126/science.1114655

Landisman, C. E., Long, M. A., Beierlein, M., Deans, M. R., Paul, D. L., & Connors, B. W. (2002). Electrical synapses in the thalamic reticular nucleus. *Journal of Neuroscience*, *22*(3), 1002–1009. https://doi.org/10.1523/jneurosci.22-03-01002.2002

Latchoumane, C. F. V., Ngo, H. V. V., Born, J., & Shin, H. S. (2017). Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms. *Neuron*, *95*(2), 424-435.e6. https://doi.org/10.1016/j.neuron.2017.06.025

Launay, P., Fleig, A., Perraud, A. L., Scharenberg, A. M., Penner, R., & Kinet, J. P. (2002a). TRPM4 is a Ca2+-activated nonselective cation channel mediating cell membrane depolarization. *Cell*. https://doi.org/10.1016/S0092-8674(02)00719-5

Le Feuvre, Y., Fricker, D., & Leresche, N. (1997). GABA(A) receptor-mediated IPSCs in rat thalamic sensory nuclei: Patterns of discharge and tonic modulation by GABA(B) autoreceptors. *Journal of Physiology*, *502*(1), 91–104. https://doi.org/10.1111/j.1469-

7793.1997.091bl.x

Lee, C. C., & Sherman, S. M. (2008). Synaptic properties of thalamic and intracortical inputs to layer 4 of the first- and higher-order cortical areas in the auditory and somatosensory systems. *Journal of Neurophysiology*, *100*(1), 317–326. https://doi.org/10.1152/jn.90391.2008

Lee, C. R., Machold, R. P., Witkovsky, P., & Rice, M. E. (2013). TRPM2 channels are required for NMDA-induced burst firing and contribute to H2O2-dependent modulation in substantia nigra pars reticulata GABAergic neurons. *Journal of Neuroscience*, *33*(3), 1157–1168. https://doi.org/10.1523/JNEUROSCI.2832-12.2013

Lee, J. H., Latchoumane, C. F. V., Park, J., Kim, J., Jeong, J., Lee, K. H., & Shin, H. S. (2019). The rostroventral part of the thalamic reticular nucleus modulates fear extinction. *Nature Communications*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-12496-9

Lee, S. C., Patrick, S. L., Richardson, K. A., & Connors, B. W. (2014). Two functionally distinct networks of gap junction-coupled inhibitory neurons in the thalamic reticular nucleus. *Journal of Neuroscience*, *34*(39), 13170–13182. https://doi.org/10.1523/JNEUROSCI.0562-14.2014

Lee, Sang Hun, Govindaiah, G., & Cox, C. L. (2007). Heterogeneity of firing properties among rat thalamic reticular nucleus neurons. *Journal of Physiology*, *582*(1), 195–208. https://doi.org/10.1113/jphysiol.2007.134254

Lee, Seung Hee, & Dan, Y. (2012). Neuromodulation of Brain States. *Neuron*, *76*(1), 209–222. https://doi.org/10.1016/j.neuron.2012.09.012

Lei, Y. T., Thuault, S. J., Launay, P., Margolskee, R. F., Kandel, E. R., & Siegelbaum, S. A. (2014). Differential contribution of TRPM4 and TRPM5 nonselective cation channels to the slow afterdepolarization in mouse prefrontal cortex neurons. *Frontiers in Cellular Neuroscience*,

8(SEP), 1–14. https://doi.org/10.3389/fncel.2014.00267

Leiva-Salcedo, E., Navarro, F., Peralta, F. A., & Riquelme, D. (2019). Cholinergic receptors activate TRPM4 in pyramidal cortical neurons at mice mPFC layer 2/3. In *The Society for Neuroscience Annual Meeting*.

Li, Y., Lopez-Huerta, V.G., Adiconis, X., Levandowski, K., Choi, S., Simmons, S.K., Arias-Garcia, M. A., Guo, B., Yao, A. Y., Blosser, T. R., Wimmer, R. D., Aida, T., Atamian, A., Naik, T., Sun, X., Bi, D., Malhotra, D., Hession, C. C., Shema, R., Gomes, M., Li., T., Hwang, E., Krol, A., Kowalczyk, M. Peça, J., Pan, G., Halassa, M. M., Levin, J. Z., Fu, Z., & Feng, G. (2020) Distinct subnetworks of the thalamic reticular nucleus. Nature (Prepublished Online). https://doi.org/10.1038/s41586-020-2504-5

Liu, X. B., & Jones, E. G. (1999). Predominance of corticothalamic synaptic inputs to thalamic reticular nucleus neurons in the rat. *Journal of Comparative Neurology*, *414*(1), 67–79. https://doi.org/10.1002/(SICI)1096-9861(19991108)414:1<67::AID-CNE6>3.0.CO;2-Z

Llinás, R. (1988). The intrinsic electrophysiological properties of mammalian nerurons: Insights into central nervous system function. *Science, 242*(3), 1654–1664.

Llinás, R., & Jahnsen, H. (1982). Electrophysiology of mammalian thalamic neurones in vitro. *Nature*, *297*(5865), 406–408. https://doi.org/10.1038/297406a0

Llinás, R. R., & Steriade, M. (2006). Bursting of thalamic neurons and states of vigilance. *Journal of Neurophysiology*, *95*(6), 3297–3308. https://doi.org/10.1152/jn.00166.2006

Long, M. A., Landisman, C. E., & Connors, B. W. (2004) Small clusters of electrically coupled neurons generate synchronus rhythms in the thalamic reticular nucleus. *Journal of Neuroscience, 24*(2), 341-349.

Lőrincz, M. L., Gunner, D., Bao, Y., Connelly, W. M., Isaac, J. T. R., Hughes, S. W., & Crunelli, V. (2015). A distinct class of slow (~0.2–2 Hz) intrinsically bursting layer 5 pyramidal neurons determines UP/DOWN state dynamics in the neocortex. *Journal of Neuroscience*, *35*(14), 5442–5458. https://doi.org/10.1523/JNEUROSCI.3603-14.2015

Lubke, J. (1993). Morphology of neurons in the thalamic reticular nucleus (TRN) of mammals as revealed by intracellular injections into fixed brain slices. *The Journal of Comparative Neurology1*, *329*(4), 458–471.

Lüthi, A. (2014). *Sleep spindles: Where they come from, what they do*. *Neuroscientist* (Vol. 20). https://doi.org/10.1177/1073858413500854

Major, G., & Tank, D. (2004). Persistent neural activity: Prevalence and mechanisms. *Current Opinion in Neurobiology*, *14*(6), 675–684. https://doi.org/10.1016/j.conb.2004.10.017

Manoach, D. S., Pan, J. Q., Purcell, S. M., & Stickgold, R. (2016). Reduced Sleep Spindles in Schizophrenia: A Treatable Endophenotype That Links Risk Genes to Impaired Cognition? *Biological Psychiatry*, *80*(8), 599–608. https://doi.org/10.1016/j.biopsych.2015.10.003

Marks, G. A., & Roffwarg, H. P. (1993). Spontaneous activity in the thalamic reticular nucleus during the sleep/wake cycle of the freely-moving rat. *Brain Research*, *623*, 241–248.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature*, *444*(7119), 610–613. https://doi.org/10.1038/nature05278

Martinez-Garcia, R. I., Voelcker, B., Zaltsman, J. B., Patrick, S. L., Stevens, T. R., Connors, B. W., & Cruikshank, S. J. (2020). Two dynamically distinct circuits driving inhibition in sensory thalamus. *Nature*,(Prepublished Online), https://doi.org/10.1038/s41586-020-2512-5.

Mathie, A. (2007). Neuronal two-pore-domain potassium channels and their regulation by G

protein-coupled receptors. *Journal of Physiology*, *578*(2), 377–385. https://doi.org/10.1113/jphysiol.2006.121582

Mccormick, D. A., & Pape, H. (1990). Properties of a hyperpolarization-activation cation current and its role in rhhythmic oscillation in thalamic relay neurons. *Journal of Physiology*, *431*, 291–318.

Mccormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neruomodulation of thalamocortical activity. *Progress in Neurobiology*, *39*, 337–388.

Mease, R. A., Metz, M., & Groh, A. (2016). Cortical Sensory Responses Are Enhanced by the Higher-Order Thalamus. *Cell Reports*, *14*(2), 208–215. https://doi.org/10.1016/j.celrep.2015.12.026

Menigoz, A., Ahmed, T., Sabanov, V., Philippaert, K., Pinto, S., Kerselaers, S., Segal, A., Freichel, M., Voets, T., Nilius, B., Vennekens, R., & Balschun, D. (2016). TRPM4-dependent post-synaptic depolarization is essential for the induction of NMDA receptor-dependent LTP in CA1 hippocampal neurons. *Pflugers Archiv European Journal of Physiology*, *468*(4), 593–607. https://doi.org/10.1007/s00424-015-1764-7

Mrejeru, A., Wei, A., & Ramirez, J. M. (2011). Calcium-activated non-selective cation currents are involved in generation of tonic and bursting activity in dopamine neurons of the substantia nigra pars compacta. *Journal of Physiology*, *589*(10), 2497–2514. https://doi.org/10.1113/jphysiol.2011.206631

Murray, S. S. (2001). Tonic and burst firing: Dual modes of thalamocortical relay. *Trends in Neurosciences*, *24*(2), 122–126. https://doi.org/10.1016/S0166-2236(00)01714-8

Nagaeva, D. V., & Akhmadeev, A. V. (2006). Structural organization, neurochemical

characteristics, and connections of the reticular nucleus of the thalamus. *Neuroscience and Behavioral Physiology*, *36*(9), 987–995. https://doi.org/10.1007/s11055-006-0134-5

Nakajima, M., Schmitt, L. I., & Halassa, M. M. (2019). Prefrontal Cortex Regulates Sensory Filtering through a Basal Ganglia-to-Thalamus Pathway. *Neuron*, *103*(3), 445-458.e10. https://doi.org/10.1016/j.neuron.2019.05.026

Neyer, C., Herr, D., Kohmann, D., Budde, T., Pape, H. C., & Coulon, P. (2016). MGluR-mediated calcium signalling in the thalamic reticular nucleus. *Cell Calcium*, *59*(6), 312–323. https://doi.org/10.1016/j.ceca.2016.03.009

Ni, K. M., Hou, X. J., Yang, C. H., Dong, P., Li, Y., Zhang, Y., Jiang, P., Berg, D. K., Duan, S., & Li, X. M. (2016). Selectively driving cholinergic fibers optically in the thalamic reticular nucleus promotes sleep. *ELife*, *5*(FEBRUARY2016), 1–17. https://doi.org/10.7554/eLife.10382

Nilius, B., Owsianik, G., Voets, T., & Peters, J. A. (2007). *Transient receptor potential cation channels in disease*. *Physiological Reviews* (Vol. 87). https://doi.org/10.1152/physrev.00021.2006

Nilius, B., Prenen, J., Droogmans, G., Voets, T., Vennekens, R., Freichel, M., Wissenbach, U., & Flockerzi, V. (2003). Voltage dependence of the Ca2+-activated cation channel TRPM4. *Journal of Biological Chemistry*, *278*(33), 30813–30820. https://doi.org/10.1074/jbc.M305127200

Nilius, B., Prenen, J., Voets, T., & Droogmans, G. (2004). Intracellular nucleotides and polyamines inhibit the Ca2+- activated cation channel TRPM4b. *Pflugers Archiv European Journal of Physiology*, *448*(1), 70–75. https://doi.org/10.1007/s00424-003-1221-x

Nuñez, A., Amzica, F., & Steriade, M. (1992). Intrinsic and synaptically generated delta (1-4 Hz) rhythms in dorsal lateral geniculate neurons and their modulation by light-induced fast (30-70 Hz) events. *Neuroscience*, *51*(2), 269–284. https://doi.org/10.1016/0306-4522(92)90314-R

O'Malley, J. J., Seibt, F., Chin, J., & Beierlein, M. (2020). TRPM4 conductances in thalamic reticular nucleus neurons generate persistent firing during slow oscillations. *The Journal of Neuroscience*, *40*(25), JN-RM-0324-20. https://doi.org/10.1523/jneurosci.0324-20.2020

Ohara, P. T., & Havton, L. A. (1996). Dendritic arbors of neurons from different regions of the rat thalamic reticular nucleus share a similar orientation. *Brain Research*, *731*(1–2), 236–240. https://doi.org/10.1016/0006-8993(96)00706-8

Ozhatil, L. C., Delalande, C., Bianchi, B., Nemeth, G., Kappel, S., Thomet, U., Ross-Kaschitza, D., Simonin, C., Rubin, M., Gertsch, J., Lochner, M., Peinelt, C., Reymons, J.-L., & Abriel, H. (2018). Identification of potent and selective small molecule inhibitors of the cation channel TRPM4. *British Journal of Pharmacology*, *175*, 2504–2519.

Paré, D., Hazrati, L. N., Parent, A., & Steriade, M. (1990). Substantia nigra pars reticulata projects to the reticular thalamic nucleus of the cat: a morphological and electrophysiological study. *Brain Research*, *535*(1), 139–146. https://doi.org/10.1016/0006-8993(90)91832-2

Parent, M., & Descarries, L. (2008). Acetylcholine innervation of the adult rat thalamus: Distribution and ultrastructural features in dorsolateral geniculate, parafascicular, and reticular thalamic nuclei. *Journal of Comparative Neurology*, *511*(5), 678–691. https://doi.org/10.1002/cne.21868

Park, J. Y., Hwang, E. M., Yarishkin, O., Seo, J. H., Kim, E., Yoo, J., Yi, G. S., Kim, D. G., & Hong, S. G. (2008). TRPM4b channel suppresses store-operated Ca2+ entry by a novel

protein-protein interaction with the TRPC3 channel. *Biochemical and Biophysical Research Communications*, *368*(3), 677–683. https://doi.org/10.1016/j.bbrc.2008.01.153

Parker, P. R. L., Cruikshank, S. J., & Connors, B. W. (2009). Stability of electrical coupling despite massive developmental changes of intrinsic neuronal physiology. *Journal of Neuroscience*, *29*(31), 9761–9770. https://doi.org/10.1523/JNEUROSCI.4568-08.2009

Pazo, J. H., Barceló, A. C., Bellantonio, E., Pazo, V. C., & Almarares, N. (2013). Electrophysiologic study of globus pallidus projections to the thalamic reticular nucleus. *Brain Research Bulletin*, *94*, 82–89. https://doi.org/10.1016/j.brainresbull.2013.02.009

Pernelle, G., Nicola, W., & Clopath, C. (2017). Gap junction plasticity can lead to spindle oscillations, 1–20. Retrieved from http://arxiv.org/abs/1710.03999

Picardo, M. C. D., Sugimura, Y. K., Dorst, K. E., Kallurkar, P. S., Akins, V. T., Ma, X., Teruyama, R., Guinamard, R., Kam, K., Saha, M. S., & Del Negro, C. A. (2019). Trpm4 ion channels in pre-bötzinger complex interneurons are essential for breathing motor pattern but not rhythm. *PLoS Biology*, *17*(2), 1–30. https://doi.org/10.1371/journal.pbio.2006094

Pigeat, R., Chausson, P., Dreyfus, F. M., Leresche, N., & Lambert, R. C. (2015). Sleep slow wave-related homo and heterosynaptic ltd of intrathalamic GABAAergic synapses: Involvement of T-type ca2+channels and metabotropic glutamate receptors. *Journal of Neuroscience*, *35*(1), 64–73. https://doi.org/10.1523/JNEUROSCI.2748-14.2015

Pinault, D. (1994). Golgi-like labeling of a single neuron recorded extracellularly. *Neuroscience Letters*, *170*, 255–260.

Pinault, D. (2003). Cellular interactions in the rat somatosensory thalamocortical system during normal epileptic 5-9 Hz oscillations. *Journal of Physiology*, *552*(3), 881–905.

https://doi.org/10.1113/jphysiol.2003.046573

Pinault, D. (2004). *The thalamic reticular nucleus: Structure, function and concept*. *Brain Research Reviews* (Vol. 46). https://doi.org/10.1016/j.brainresrev.2004.04.008

Pinault, D., & Deschenes, M. (1998). Anatomical evidence for a mechanism of lateral inhibition in the rat thalamus. *European Journal of Neuroscience*, *10*(11), 3462–3469. https://doi.org/10.1046/j.1460-9568.1998.00362.x

Pinault, D., & Deschênes, M. (1998). Projection and innervation patterns of individual thalamic reticular axons in the thalamus of the adult rat: A three-dimensional, graphic, and morphometric analysis. *Journal of Comparative Neurology*, *391*(2), 180–203. https://doi.org/10.1002/(SICI)1096-9861(19980209)391:2<180::AID-CNE3>3.0.CO;2-Z

Pita-Almenar, J. D., Yu, D., Lu, H.-C., & Beierlein, M. (2014a). Cholinergic synaptic inputs triggers thalamic network activity. In *The Society for Neuroscience Annual Meeting*.

Pita-Almenar, J. D., Yu, D., Lu, H. C., & Beierlein, M. (2014b). Mechanisms Underlying Desynchronization of Cholinergic-Evoked Thalamic Network Activity. *Journal of Neuroscience*, *34*(43), 14463–14474. https://doi.org/10.1523/JNEUROSCI.2321-14.2014

Pressler, R. T., & Regehr, W. G. (2013). Metabotropic glutamate receptors drive global persistent inhibition in the visual thalamus. *Journal of Neuroscience*, *33*(6), 2494–2506. https://doi.org/10.1523/JNEUROSCI.3458-12.2013

Raeva, S., & Lukashev, A. (1993). Unit activity in human thalamic reticularis neurons. II. Activity evoked by significant and non-significant verbal or sensory stimuli. *Electroencephalography and Clinical Neurophysiology*, *86*(2), 110–122. https://doi.org/10.1016/0013-4694(93)90083-8

Reinagel, P., Godwin, D., Sherman, S. M., & Koch, C. (1999). Encoding of visual information by

LGN bursts. *Journal of Neurophysiology*, *81*(5), 2558–2569. https://doi.org/10.1152/jn.1999.81.5.2558

Riquelme, D., Silva, I., Philp, A. M., Huidobro-Toro, J. P., Cerda, O., Trimmer, J. S., & Leiva-Salcedo, E. (2018). Subcellular localization and activity of TRPM4 in medial prefrontal cortex layer 2/3. *Frontiers in Cellular Neuroscience*, *12*(January), 1–13. https://doi.org/10.3389/fncel.2018.00012

Ritter-Makinson, S., Clemente-Perez, A., Higashikubo, B., Cho, F. S., Holden, S. S., Bennett, E., Chkhaidze, A., Rooda, O. H. J. E., Cornet, M.-C., Hoebeek, F. E., Yamakawa, K., Cilio, M. R., Delord, B., & Paz, J. T. (2019). Augmented Reticular Thalamic Bursting and Seizures in Scn1a-Dravet Syndrome. *Cell Reports*, *26*(1), 54-64.e6. https://doi.org/10.1016/j.celrep.2018.12.018

Rodríguez, J. J., Noristani, H. N., Hoover, W. B., Linley, S. B., & Vertes, R. P. (2011). Serotonergic projections and serotonin receptor expression in the reticular nucleus of the thalamus in the rat. *Synapse*, *65*(9), 919–928. https://doi.org/10.1002/syn.20920

Ross, D. T., Graham, D. I., & Adams, J. H. (1993). Selective loss of neurons from the thalamic reticular nucleus following sever human head injury. *Journal of Neurotrauma*, *10*(2), 151–165. https://doi.org/10.1089/neu.1993.10.151

Sachdeva, R., Schlotterer, A., Schumacher, D., Matka, C., Mathar, I., Dietrich, N., Medert, R., Kriebs, U., Lin, J., Nawroth, P., Birnbaumer, L., Fleming, T., Hammes, H. P., & Freichel, M. (2018). TRPC proteins contribute to development of diabetic retinopathy and regulate glyoxalase 1 activity and methylglyoxal accumulation. *Molecular Metabolism*, *9*(January), 156–167. https://doi.org/10.1016/j.molmet.2018.01.003

Sanchez-Vives, M. V., & McCormick, D. A. (2000). Cellular and network mechanisms of rhytmic recurrent activity in neocortex. *Nature Neuroscience*, *3*(10), 1027–1034. https://doi.org/10.1038/79848

Scaglione, A., & Moxon, K. A. (2006). Behaviorally modulated filter model for the thalamic reticular nucleus. *Annual International Conference of the IEEE Engineering in Medicine and Biology - Proceedings*, 595–598. https://doi.org/10.1109/IEMBS.2006.260583

Scheibel, M. E., & Scheibel, A. B. (1972). Specialized Organizational Reticularis Patterns Thalami within the the Nucleus of. *Experimental Neurology*, *34*, 316–322.

Schiffmann, S. N., & Vanderhaegen, J. J. (1991). Distribution of cells containing mRNA encoding cholecystokinin in the rat central nervous system. *The Journal of Comparative Neurology*, *304*(2), 219–233.

Schofield, C. M., Kleiman-Weiner, M., Rudolph, U., & Huguenard, J. R. (2009). A gain in GABA A receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(18), 7630–7635. https://doi.org/10.1073/pnas.0811326106

Shosaku, A., & Sumitomo, I. (1983). Auditory neurons in the rat thalamic reticular nucleus. *Experimental Brain Research*, *49*, 432–442.

Sieber, A. R., Min, R., & Nevian, T. (2013). Non-hebbian long-term potentiation of inhibitory synapses in the thalamus. *Journal of Neuroscience*, *33*(40), 15675–15685. https://doi.org/10.1523/JNEUROSCI.0247-13.2013

Sohal, V. S., Huntsman, M. M., & Huguenard, J. R. (2000). Reciprocal inhibitory connections produce desynchronizing phase lags during intrathalamic oscillations. *Neurocomputing*, *32–*

33(5), 509–516. https://doi.org/10.1016/S0925-2312(00)00206-X

Söhl, G., Maxeiner, S., & Willecke, K. (2005). Expression and functions of neuronal gap junctions. *Nature Reviews Neuroscience*, *6*(3), 191–200. https://doi.org/10.1038/nrn1627

Spreafico, R., Battaglia, G., & Frassoni, C. (1991). The reticular thalamic nucleus (RTN) of the rat: Cytoarchitectural, Golgi, immunocytochemical, and horseradish peroxidase study. *The Journal of Comparative Neurology*, *304*(3), 478–490.

Spreafico, R., de Curtis, M., Frassoni, C., & Avanzini, G. (1988). Electrophysiological characteristics of morphologically identified reticular thalamic neurons from rat slices. *Neuroscience*, *27*(2), 629–638. https://doi.org/10.1016/0306-4522(88)90294-1

Steriade, M., Contreras, D., Dossi, R. C., & Nunez, A. (1993). The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: Scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *Journal of Neuroscience*, *13*(8), 3284–3299. https://doi.org/10.1523/jneurosci.13-08-03284.1993

Steriade, M., Deschenes, M., Domich, L., & Mulle, C. (1985). Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *Journal of Neurophysiology*, *54*(6), 1473–1497. https://doi.org/10.1152/jn.1985.54.6.1473

Steriade, M., Domich, L., & Oakson, G. (1986). Reticularis thalami neurons revisited: Activity changes during shifts in states of vigilance. *Journal of Neuroscience*, *6*(1), 68–81. https://doi.org/10.1523/jneurosci.06-01-00068.1986

Steriade, M., Timofeev, I., & Grenier, F. (2001). Natural waking and sleep states: A view from inside neocortical neurons. *Journal of Neurophysiology*, *85*(5), 1969–1985. https://doi.org/10.1152/jn.2001.85.5.1969

Steriade, Mircea, & Amzica, F. (1996). Intracortical and corticothalamic coherency of fast spontaneous oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(6), 2533–2538. https://doi.org/10.1073/pnas.93.6.2533

Steriade, Mircea, McCormick, D. A., & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science*, *262*(5134), 679–685. https://doi.org/10.1126/science.8235588

Stocker, M., & Pedarzani, P. (2000). Differential distribution of three Ca2+-activated K2+ channel subunits, SK1, SK2, and SK3, in the adult rat central nervous system. *Molecular and Cellular Neurosciences*, *15*(5), 476–493. https://doi.org/10.1006/mcne.2000.0842

Sun, Y.-G., Pita-Almenar, J. D., Wu, C.-S., Renger, J. J., Uebele, V. N., Lu, H.-C., & Beierlein, M. (2013). Biphasic cholinergic synaptic transmission controls action potential activity in thalamic reticular nucleus neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(5), 2048–2059. https://doi.org/10.1523/JNEUROSCI.3177-12.2013

Sun, Y. G., Rupprecht, V., Zhou, L., Dasgupta, R., Seibt, F., & Beierlein, M. (2016). MGluR1 and mGluR5 synergistically control cholinergic synaptic transmission in the thalamic reticular nucleus. *Journal of Neuroscience*, *36*(30), 7886–7896. https://doi.org/10.1523/JNEUROSCI.0409-16.2016

Swadlow, H. A., & Gusev, A. G. (2001). The impact of "bursting" thalamic impulses at a neocortical synapse. *Nature Neuroscience*, *4*(4), 402–408. https://doi.org/10.1038/86054

Tian, J., & Zhu, M. (2018). GABAB Receptors Augment TRPC3-Mediated Slow Excitatory Postsynaptic Current to Regulate Cerebellar Purkinje Neuron Response to Type-1

Metabotropic Glutamate Receptor Activation. *Cells*, *7*(8), 90. https://doi.org/10.3390/cells7080090

Ting, J. T., Daigle, T., Chen, Q., & Feng, G. (2014). Acute brain slice method for adult and aging animals: application of targeted patch clamp analysis and optogenetics. In M. Martina & S. Taverna (Eds.), *Methods in Molecular Biology* (Patch-Clam, Vol. 1183, pp. 221–242). New York: Humana Press. https://doi.org/10.1007/978-1-4939-1096-0

Tremblay, R., Lee, S., & Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*, *91*(2), 260–292. https://doi.org/10.1016/j.neuron.2016.06.033

Turner, R. W., Kruskic, M., Teves, M., Scheidl-Yee, T., Hameed, S., & Zamponi, G. W. (2014). Neuronal expression of the intermediate conductance calcium-activated potassium channel KCa3.1 in the mammalian central nervous system. *Pflugers Archiv European Journal of Physiology*, *467*(2), 311–328. https://doi.org/10.1007/s00424-014-1523-1

Turner, R. W., & Zamponi, G. W. (2014). T-type channels buddy up. *Pflugers Archiv European Journal of Physiology*, *466*(4), 661–675. https://doi.org/10.1007/s00424-013-1434-6

Ulrich, D., Lalanne, T., Gassmann, M., & Bettler, B. (2018). GABAB receptor subtypes differentially regulate thalamic spindle oscillations. *Neuropharmacology*, *136*, 106–116. https://doi.org/10.1016/j.neuropharm.2017.10.033

Urbain, N., Fourcaud-Trocmé, N., Laheux, S., Salin, P. A., & Gentet, L. J. (2019) Brain-state-dependent modulation of neuronal firing and membrane potential dynamics in the somatosensory thalamus during natural sleep. *Cell Reports, 26*(6), 1443-1457.

Vantomme, G., Osorio-Forero, A., Lüthi, A., & Fernandez, L. M. J. (2019). Regulation of local

sleep by the thalamic reticular nucleus. *Frontiers in Neuroscience*, *13*(JUN), 1–8. https://doi.org/10.3389/fnins.2019.00576

Varga, A. W., Wohlleber, M. E., Giménez, S., Romero, S., Alonso, J. F., Ducca, E. L., … Osorio, R. S. (2016). Reduced Slow-Wave Sleep Is Associated with High Cerebrospinal Fluid Aβ42 Levels in Cognitively Normal Elderly. *Sleep*, *39*(11), 2041–2048. https://doi.org/10.5665/sleep.6240

Viviano, J. D., & Schneider, K. A. (2015). Interhemispheric interactions of the human thalamic reticular nucleus. *Journal of Neuroscience*, *35*(5), 2026–2032. https://doi.org/10.1523/JNEUROSCI.2623-14.2015

Wahl-Schott, C., & Biel, M. (2009). HCN channels: Structure, cellular regulation and physiological function. *Cellular and Molecular Life Sciences*, *66*(3), 470–494. https://doi.org/10.1007/s00018-008-8525-0

Wang, S., Bickford, M. E., Van Horn, S. C., Erisir, A., Godwin, D. W., & Sherman, S. M. (2001). Synaptic targets of thalamic reticular nucleus terminals in the visual thalamus of the cat. *Journal of Comparative Neurology*, *440*(4), 321–341. https://doi.org/10.1002/cne.1389

Weese, G. D., Phillips, J. M., & Brown, V. J. (1999). Attentional orienting is impaired by unilateral lesions of the thalamic reticular nucleus in the rat. *Journal of Neuroscience*, *19*(22), 10135–10139. https://doi.org/10.1523/jneurosci.19-22-10135.1999

Weiser, M., Bueno, E., Sekirnjak, C., Marione, M. E., Baker, H., Hillman, D., Chen, S., Thornhill, W., Ellisman, M., & Rudy, B. (1995). The potassium channel subunit KV3.1b is localized to somatic and axonal membranes of specific populations of CNS neurons. *Journal of Neuroscience*, *15*(6), 4298–4314. https://doi.org/10.1523/jneurosci.15-06-04298.1995

Weiser, Michael, Vega-Saenz De Miera, E., Kentros, C., Moreno, H., Franzen, L., Hillman, D., Baker, H., & Rudy, B. (1994). Differential expression of Shaw-related K+ channels in the rat central nervous system. *Journal of Neuroscience*, *14*(3 I), 949–972. https://doi.org/10.1523/jneurosci.14-03-00949.1994

Wells, M. F., Wimmer, R. D., Schmitt, L. I., Feng, G., & Halassa, M. M. (2016). Thalamic reticular impairment underlies attention deficit in Ptchd1 Y'mice. *Nature*, *532*(7597), 58–63. https://doi.org/10.1038/nature17427

Weyand, T. G., Boudreaux, M., & Guido, W. (2001). Burst and tonic response modes in thalamic neurons during sleep and wakefulness. *Journal of Neurophysiology*, *85*(3), 1107–1118. https://doi.org/10.1152/jn.2001.85.3.1107

Williams, S. R., Tóth, T. I., Turner, J. P., Hughes, S. W., & Crunelli, V. (1997). The "window" component of the low threshold Ca2+ current produces input signal amplification and bistability in cat and rat thalamocortical neurones. *Journal of Physiology*. https://doi.org/10.1111/j.1469-7793.1997.689ba.x

Williamson, A. M., Ohara, P. T., Ralston, D. D., Milroy, A. M., & Ralston, H. J. (1994). Analysis of gamma-aminobutyric acidergic synaptic contacts in the thalamic reticular nucleus of the monkey. *Journal of Comparative Neurology*, *349*(2), 182–192. https://doi.org/10.1002/cne.903490203

Wimmer, R. D., Astori, S., Bond, C. T., Rovó, Z., Chatton, J. Y., Adelman, J. P., Franken, P., & Lüthi, A. (2012). Sustaining sleep spindles through enhanced SK2-channel activity consolidates sleep and elevates arousal threshold. *Journal of Neuroscience*, *32*(40), 13917–13928. https://doi.org/10.1523/JNEUROSCI.2313-12.2012

Wimmer, R. D., Schmitt, L. I., Davidson, T. J., Nakajima, M., Deisseroth, K., & Halassa, M. M. (2015). Thalamic control of sensory selection in divided attention. *Nature*, *526*(7575), 705–709. https://doi.org/10.1038/nature15398

Woo, S. K., Kwon, M. S., Ivanov, A., Gerzanich, V., & Simard, J. M. (2013). The sulfonylurea receptor 1 (Sur1)-transient receptor potential melastatin 4 (Trpm4) channel. *Journal of Biological Chemistry*, *288*(5), 3655–3667. https://doi.org/10.1074/jbc.M112.428219

Yamaguchi, S., Tanimoto, A., Otsuguro, K. I., Hibino, H., & Ito, S. (2014). Negatively charged amino acids near and in transient receptor potential (TRP) domain of TRPM4 channel are one determinant of its Ca2+ sensitivity. *Journal of Biological Chemistry*, *289*(51), 35265–35282. https://doi.org/10.1074/jbc.M114.606087

Zaman, T., Lee, K., Park, C., Paydar, A., Choi, J. H., Cheong, E., Lee, C. J., & Shin, H. S. (2011). CaV2.3 Channels Are Critical for Oscillatory Burst Discharges in the Reticular Thalamus and Absence Epilepsy. *Neuron*, *70*(1), 95–108. https://doi.org/10.1016/j.neuron.2011.02.042

Zeisel, A., Muñoz-Manchado, Ana B. Codeluppi, Simone Lönnerberg, P., La Manno, G., Juréus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., Rolny, C., Castelo-Branco, G., Hjerling-Leffler, J., & Linnarsson, S. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, *347*(6226), 1138–1141.

Zhang, S. J., Huguenard, J. R., & Prince, D. A. (1997). GABA(A) receptor-mediated Cl- currents in rat thalamic reticular and relay neurons. *Journal of Neurophysiology*, *78*(5), 2280–2286. https://doi.org/10.1152/jn.1997.78.5.2280

Zhang, Zheng, Okawa, H., Wang, Y., & Liman, E. R. (2005). Phosphatidylinositol 4,5-Bisphosphate rescues TRPM4 channels from desensitization. *Journal of Biological Chemistry*,

280(47), 39185–39192. https://doi.org/10.1074/jbc.M506965200

Zhang, Zizhen, Reboreda, A., Alonso, A., Barker, P. A., & Séguéla, P. (2011). TRPC channels underlie cholinergic plateau potentials and persistent activity in entorhinal cortex. *Hippocampus*, *21*(4), 386–397. https://doi.org/10.1002/hipo.20755

Zhang, Zizhen, & Séguéla, P. (2010). Metabotropic induction of persistent activity in layers II/III of anterior cingulate cortex. *Cerebral Cortex*, *20*(12), 2948–2957. https://doi.org/10.1093/cercor/bhq043

Zhao, S., Ting, J. T., Atallah, H. E., Qiu, L., Tan, J., Gloss, B., Augustine, G. J., Deisseroth, K., Luo, M., Graybiel, A. M., & Feng, G. (2011). Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nature Methods*, *8*(9), 745–755. https://doi.org/10.1038/nmeth.1668

Zikopoulos, B., & Barbas, H. (2012). Pathways for emotions and attention converge on the thalamic reticular nucleus in primates. *Journal of Neuroscience*, *32*(15), 5338–5350. https://doi.org/10.1523/jneurosci.4793-11.2012

Zylberberg, J., & Strowbridge, B. W. (2017). Mechanisms of persistent activity in cortical circuits: Possible neural substrates for working memory. *Annual Review of Physiology*, *40*, 60. – 627. https://doi.org/10.1016/j.physbeh.2017.03.040

Vita

John Joseph (JJ) O'Malley was born in Virginia, the son of John and Marie O'Malley. After graduating from Bishop Dennis J O'Connell High School he entered James Madison University in Harrisonburg, Virginia. He received the degree of Bachelor of Arts with a double major in philosophy and psychology in 2012 and the degree of Master of Arts in experimental psychology in 2014. The following year he worked as a laboratory technician in the Department of Neurobiology at the University of Alabama at Birmingham. In August of 2015 he entered the University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences in Houston.