Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

David Aaron Nicholson

Cerebellothalamic and thalamostriatal projections in a songbird, the Bengalese Finch

By

David Nicholson

Doctor of Philosophy

Neuroscience

_____ [Advisor's signature]
Samuel J. Sober
Advisor

_____ [Member's signature]
Ellen Hess
Committee Member

_____ [Member's signature]
Dieter Jaeger
Committee Member

_____ [Member's signature]
Robert Liu
Committee Member

_____ [Member's signature]
Javier Medina
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

Cerebellothalamic and thalamostriatal projections in a songbird, the Bengalese Finch

By

David Nicholson

Bachelor of Science, Biology

Advisor: Samuel J. Sober, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Neuroscience

2017

Abstract

Cerebellothalamic and thalamostriatal projections in a songbird, the Bengalese Finch

By David Nicholson

Like a child learning to speak, songbirds learn their songs as juveniles from adult tutors. They then perfect their song by practicing it thousands of times during development. This behavior makes songbirds an excellent model system through which neuroscientists can understand how the brain learns and produces a motor skill that resembles speech. Birdsong is under the control of a network of nuclei in the songbird brain known as the song system. To what extent is the song system like motor systems in the mammalian brain? Many studies have shown how a thalamocortical-basal ganglia loop in the song system known as the Anterior Forebrain Pathway (AFP) contributes to learning song and adaptively maintaining it in adulthood. In mammals, the basal ganglia receive input from the thalamus, including thalamic regions that receive output from the cerebellum. It is unknown whether the basal ganglia nucleus of the song system, Area X, receives thalamic input, or whether such inputs might be used to convey cerebellar outputs to the song system. I first demonstrate that the cerebellum projects to dorsal thalamus in the Bengalese finch, and provide the first detailed description of the cerebellothalamic projections. Then, using a viral vector that specifically labels presynaptic axon terminals, I demonstrate that dorsal thalamus projects to the striatum. To determine the sources and targets of the thalamostriatal system in songbirds, I use tracers to map thalamic regions and immunohistochemistry to identify song system nuclei. I find that DLM and immediately adjacent DT_{CbN} project to Area X. In contrast, more medial and ventral subregions of DT_{CbN} project to striatum outside Area X. These results suggest that in the AFP the basal ganglia integrates feedback from the thalamic region to which it projects as well as input from thalamic regions that receive cerebellar output, much like the mammalian basal ganglia.

Cerebellothalamic and thalamostriatal projections in a songbird, the Bengalese Finch

By

David Nicholson

Bachelor of Science, Biology

Advisor: Samuel J. Sober, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Neuroscience
2017

Acknowledgements

Mushy stuff:
Thank you to my parents and my brother for always letting me by myself and find my own way. We know I am so hardheaded that you couldn't have stopped me if you'd tried. Thank you to my sister, Angie, and your family, as well as my Aunt Beth and Uncle Bob and the rest of the Felkel clan; I am so fortunate to have had family nearby to support me during graduate school. You gave me a place to live when I got here and you fed me and reminded me there was a world outside the lab. Muchas gracias a mi otra familia también, Vidal y Flory y toda esa familia Cotorra, nunca voy a olvidar las aventuras y travesuras que vivimos juntos. Thank you to the people that helped me survive even when I wasn't sure that I wanted to. I will spend a lifetime trying to be as good as you have been to me.

Mushy science stuff:
Thank you, Sam, for being the best boss I have ever had. Thank you to all members of the Sober lab, past and present: you are my science family. Sorry about all the bad jokes. Thanks also to my extended science family in the Biology department, the Neuroscience graduate program, and at Emory. There's more of you than I can list and I couldn't have done this without you.
Last but not least, thank you to my committee for keeping me on track to graduate, and for pushing me to write this thesis. If it's worth reading, that's because of you. I'll take the blame for any remaining typos.

Table of Contents

List of Figures and Tables

Please see also the supplementary information:

https://doi.org/10.6084/m9.figshare.5437975

List of Abbreviations

Abbreviations

AC	anterior commissure
CbI	intermediate cerebellar nucleus
CbL	lateral cerebellar nucleus
CbM	medial cerebellar nucleus
CbN	cerebellar nuclei
DIP	dorsointermediate thalamus, posterior part
DLI	dorsolateral thalamus, intermediate part
DLL	dorsolateral thalamus, lateral part
DLP	dorsolateral thalamus, posterior part
GP	Globus pallidus
HA	Accessory hyperpallium
Hb	habenula
HVC	Used as a proper name (Reiner, Perkel et al. 2004)
InC	Interstitial nucleus of Cajal
LMAN	lateral magnocellular nucleus of anterior nidopallium
meso	mesopallim
MMAN	medial magnocellular nucleus of anterior nidopallium
MSt	medial striatum
NI	intermediate nidopallium

Nido	nidopallium
NOv	nucleus ovoidalis
OM	occipitomesencephalic tract
PC	posterior commissure
PSL	pallial-subpallial lamina (the dividing line between cortex and the basal ganglia)
PT	pretectal nucleus
Rt	nucleus rotundus
sDMP	songbird dorsomedial thalamus, posterior part
sDLM	songbird dorsolateral thalamus, medial part
SpL	lateral spiriform nucleus
SpM	medial spiriform nucleus
Uva	uvaeform nucleus
VIA	ventrointermediate area

I Introduction

Much like children learning to talk, songbirds learn their song from an adult (Marler and Tamura 1964, Doupe and Kuhl 1999, Bolhuis, Okanoya et al. 2010). As juveniles, songbirds spend weeks or months practicing until their vocalizations sound like adult song and they can produce vocalizations with minimal trial-to-trial variability (Tchernichovski, Mitra et al. 2001). Because of this, songbirds provide neuroscience with a model system for understanding how the brain learns and produces motor skills like speaking a language, playing the piano, or swinging a golf club. One of the advantages of songbirds as a model system is that the brain regions required for learning and producing song are largely separate from the rest of the brain. Song is under control of a network of nuclei in the songbird brain known as the song system (Nottebohm, Stokes et al. 1976, Brenowitz, Margoliash et al. 1997). Although the song system is found only in songbird brains, it is thought to have evolved from brain regions shared by all vertebrates, and it is now clear that the songbird brain contains all major regions involved in motor control in humans, such as the cortex, the basal ganglia, and the cerebellum (Reiner, Perkel et al. 2004). The neuroanatomy of the song system has been extensively studied, but there are important unanswered questions about its structure (Gale and Perkel 2010) and how similar its structure is to motor systems in mammals. Specifically, it is not known whether the thalamus projects to the basal ganglia in the song system, and it is not known whether an anatomical pathway exists from the cerebellum through thalamus to the forebrain nuclei of the song system, as might be expected based on our understanding of mammalian motor systems. The answer to these questions will inform our understanding of how motor systems function.

In this Introduction I first explain why songbirds provide an excellent model of speech and similar motor skills. Then I give an overview of motor systems in mammals and the song system in songbirds. I provide a detailed review of the anatomy, physiology, and theories of function of the basal ganglia. This review compares findings from mammals with results from studies of a thalamocortical-basal ganglia loop in the song system of songbirds. I focus on what role thalamostriatal and cerebellothalamic projections in the song system would play given current models of the function of this thalamocortical-basal ganglia loop. To the same end, I review literature on thalamostriatal system and cerebellothalamic projections in mammals, and any existing relevant results from studies of birds. I also describe a network of avian brain regions that has been proposed as the homolog of the descending motor systems in mammals, and some related "non-canonical" pathways in the song system. As I hope will become clear, these previous studies and the results in this dissertation have important implications for the extent to which the song system resembles mammalian motor systems, and provide a starting point to answer the question of where and how the song system interacts with other motor systems in the songbird brain. Lastly I outline the studies performed that are described in this dissertation.

I.1 Songbirds as a model system

I.1.A Parallels between birdsong, speech, and similar motor skills

Speech and birdsong share many characteristics (Doupe and Kuhl 1999, Bolhuis, Okanoya et al. 2010). For the purposes of this Introduction, I will divide those shared characteristics into two categories: **sensorimotor learning** and **social interaction**.

I.1.A.1 Sensorimotor learning

Sensorimotor learning is defined as an improvement in execution of a motor skill that results from the sensory feedback provided by practicing the motor skill (Krakauer and Mazzoni 2011). As this definition emphasizes, sensory feedback plays a crucial role in this sort of motor learning. For example, humans and songbirds require auditory feedback (Cowie and Douglas-Cowie 1992, Brainard and Doupe 2000) and somatosensory feedback (Tremblay, Shiller et al. 2003, Elemans 2014) to acquire and to maintain their vocalizations. Two theoretical frameworks for how the brain uses sensory feedback during movement that pertain to the work in this dissertation are **reinforcement learning** (Sutton and Barto 1998) and **internal models** (Francis and Wonham 1976).

Most models of how songbirds acquire their song and maintain its quality throughout life have focused on reinforcement learning. This framework formalizes a simple learning rule: if an action tends to be followed by reward in a certain context, then the likelihood of producing that action in the same context should be reinforced (Fee 2012). Such models have mapped reinforcement learning algorithms onto the structure of a thalamocortical basal-ganglia loop in the song system known as the anterior forebrain pathway (Doya and Sejnowski 1998, Troyer and Doupe 2000, Troyer and Doupe 2000, Fee and Goldberg 2011). In section I.2.A.3.b I review the structure of the Anterior Forebrain Pathway and in section I.2.A.3.f I review one hypothesis about how it functions based on reinforcement learning models.

Unlike reinforcement learning, the internal model framework has formed the basis of only a few studies of sensorimotor learning in songbirds (Hanuschkin, Ganguli et al. 2013, Giret, Kornfeld et al. 2014). Many studies of the cerebellum, however, have framed the results in terms of internal models (Kawato 1999). The concept of internal models comes from the field of control theory, that typically studies how best to design some controller of a system so that the output of the system matches the control signal. One theorem from control theory states that any good "regulator", i.e., controller, must contain an explicit model (Conant and Ross Ashby 1970). This was proven mathematically for one type of linear system (Francis and Wonham 1976) in the study that gave rise to the term "internal model". In this context, an internal model is a mapping from the command signal to the predicted output. Many researchers studying motor systems assume the brain must build internal models of the body it controls. One common experimental approach to studying internal models is to impose a persistent change in sensory feedback and study the resulting long-term changes in motor output, a process known as adaptation (Bastian 2006) Adaptation has been studied extensively, including in speech (Houde and Jordan 1998, Jones and Munhall 2000) and in birdsong (Sober and Brainard 2009, Sober and Brainard 2012). In terms of the cerebellum, it is theorized that the connections of this brain structure are suitable for computing an internal model and updating the model when sensory feedback errors arise, as I review in section I.2.A.5. Although no published study implicates the cerebellum in birdsong, a significant body of work points the cerebellum as a major brain region required for adaptation (Ito 1982, Martin, Keating et al. 1996, Martin, Keating et al. 1996, Bastian 2006).

It should also be recognized that there are other computations thought to contribute to motor learning in addition to reinforcement learning and internal models. For example, explicit knowledge of sensory perturbation accelerates adaptation (Taylor, Krakauer et al. 2014). Explicit knowledge is probably localized to other brain regions such as cerebral cortex (Taylor and Ivry 2014), and is less relevant to the work in this dissertation, so it will not be discussed further.

I.1.A.2 Social interaction

Social interactions have a tremendous impact on learning speech (Hoff 2006, Tomasello 2009), birdsong (Marler and Tamura 1964, Marler 1970, Marler 1970, Nelson, Marler et al. 1995, Beecher and Burt 2004, Beecher and Brenowitz 2005, Derégnaucourt, Poirier et al. 2013) , and other motor skills acquired in part by imitation. While there is an established network of brain areas related to social behaviors in vertebrates (Newman 1999, Goodson 2005), these networks are not conceived of as including sensorimotor areas in the basal ganglia or the cerebellum. By the same token, models of motor learning in the basal ganglia and cerebellar such as those described in the preceding section do not typically include an explicit role for social interaction. However evidence exists that the basal ganglia and cerebellum are involved with processing social interactions that relate to motor learning. A significant body of work demonstrates that social context influences neural activity within a thalamocortical-basal ganglia pathway of the song system (Kao and Brainard 2006, Yanagihara and Hessler 2006, Stepanek and Doupe 2010). I discuss how these findings have contributed to current understandings of basal ganglia function in section I.2.A.3. Again to my knowledge no current models explicitly include social interaction as a separate input to the model, for example to ask how social interaction interacts with input to a model from sensory

feedback. With respect to the cerebellum, it has been implicated in autism (Fatemi, Aldinger et al. 2012), a syndrome characterized by impairments in social cognition and speech acquisition. Of particular relevance here is the hypothesis that there is a sensitive period of development during which the cerebellum is required for other brain areas to acquire behaviors via social interaction (Wang, Kloth et al. 2014). Songbirds provide an excellent model system to carry out controlled experiments to investigate the sensitive period (Beecher and Brenowitz 2005). In the Discussion (section IV.2.B.8) of the discussion I outline experiments to investigate whether the cerebellum is involved with socially-guided imitation in songbirds.

I.2 The song system as a general model of motor systems

As just described, both song and speech are behaviors that the brain acquires via sensorimotor learning and social interaction. These parallels between song and speech raise the possibility that understanding how the songbird brain learns and produces song could contribute to our understanding of how the human brain learns and produces speech and similar motor skills. But understanding the neural basis of song might not be very informative if the songbird brain is very different from the human brain. Comparative neuroanatomy has given us good reason to think that the avian and mammalian brains are actually quite similar. Around the turn of the nineteenth century, comparative neuroanatomists viewed the brains of birds (songbirds as well as non-songbird species) as lacking neocortex but having evolved additional "layers" of the basal ganglia (Kappers, Huber et al. 1936). However, modern neuroscientific methods have revealed that the same regions are found in the bird brain that are found in the brain of humans and all other vertebrates (Jarvis, Gunturkun et al. 2005). The avian brain does contain basal ganglia, and there

are cortical regions dorsal to the basal ganglia (although only a small portion of the cortical region resembles the six-layered neocortex seen in mammals). Other areas typically thought of as part of the motor system are also present, including the cerebellum and a motor cortex-like region that is the source of descending projections onto motor neurons in the brain stem and spinal cord. Given the 300 million years since their evolutionary paths diverged (Kumar and Hedges 1998), there are of course differences between mammalian and bird brains, and the goal of the work described in this dissertation is to address some of the questions about how different those motor systems are.

Specifically the work in this dissertation investigates whether there is a thalamostriatal projection in the song system, and whether there is a pathway for cerebellar output to reach the song system through the thalamus. If these connections do exist in the song system, what is their function? So that I can address this question of function in the discussion, I provide an extensive review here. I begin with a high-level overview of motor systems in mammals and of the song system in songbirds. I then review anatomy, physiology, and theoretical models of the basal ganglia in mammals and in the song system of songbirds. The reason I start with the basal ganglia is because both of the questions addressed by work in this dissertation are related to a thalamocortical-basal ganglia loop in the song system known as the Anterior Forebrain Pathway. I present one hypothesis about the function of the anterior forebrain pathway, built within a reinforcement learning framework, so that I can discuss how the results in this dissertation might change that model. In addition, I give an in-depth review of current understanding of thalamus in songbirds, especially places in the literature that suggest natural follow-up experiments to the studies in this dissertation. I then move on to review what is known about the thalamostriatal system and cerebellothalamic

pathways in mammals. In addition, I review other pathways through which the cerebellum might be involved with vocalization. I summarize speech motor control pathways in terms of mammalian motor systems described. I also review what is known about projections of the cerebellar nuclei in birds and the evidence for pathways that the cerebellum might influence vocalization in birds. Lastly, I describe a pyramidal-tract like pathway in the songbird brain that like the descending motor pathways in mammals is interconnected with the cerebellum. Previous work on this avian pyramidal tract suggests it might be part of the motor system that controls muscles not limited to the song system, such as jaw muscles that open the beak, and hence would be a place that the song system and other motor systems interact.

I.2.A.1 The motor system in mammals

For the purposes of this Introduction, I adopt the following definition of a motor skill: an action the brain learns to perform after significant practice, and that it can then execute in a flexible way so as to achieve a constant outcome in different environments (Dudman and Krakauer 2016). One example of a motor skill would be swinging a bat to hit a baseball, and another example would be speaking a language. A reflex would not be a motor skill because it does not require practice even though the brain may adapt a reflex to different environments. Other examples of motor skills are the reaching and grasping tasks used in experiments with nonhuman primates to understand function of motor cortex via single-unit recordings—many such studies will be referenced below. Most studies of motor skills (as just defined) in mammals assume that motor cortical regions play a key role in execution, as shown in Figure 1. These studies further assume that the basal ganglia

and the cerebellum contribute to motor skills by computing distinct algorithms that each area is specialized to carry out (Doya 1999, Doya 2000, Shmuelof and Krakauer 2011, Dudman and Krakauer 2016). Both subcortical areas then output the results of their computations to motor thalamus (Ghez and Krakauer 2000, Bosch-Bouju, Hyland et al. 2013), the thalamic area that projects to motor cortical regions. In turn the motor cortical regions integrate output from the basal ganglia and cerebellum, as communicated by motor thalamus, so that it can better execute the motor skill (Ghez and

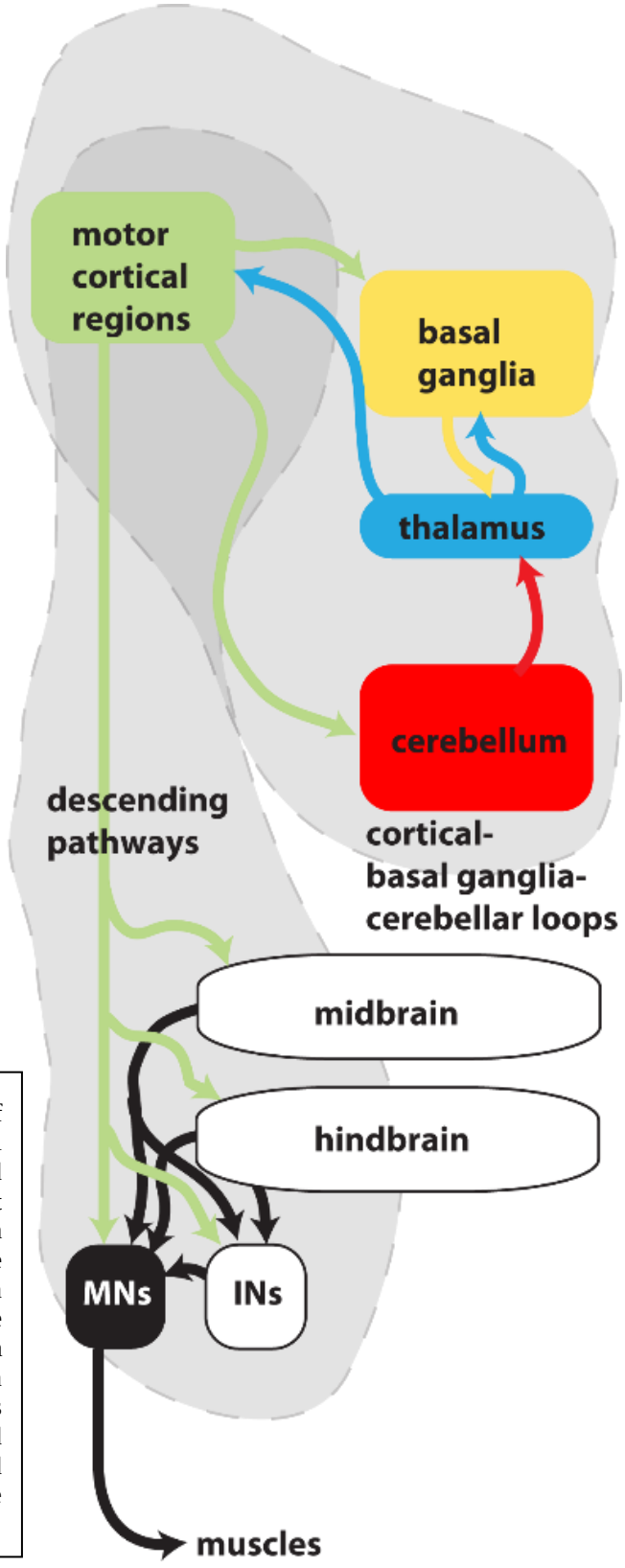

Figure 1. Mammalian motor systems. Many studies of mammalian motor systems assume that motor cortical regions (green) play a key role in executing learned motor skills because these regions alone modulate not just the MNs that control muscles but also midbrain and hindbrain regions that themselves modulate ongoing MN activity (the descending pathways, green arrows). Furthermore, cortex alone integrates the output of the basal ganglia (yellow) and the cerebellum (red), two structures known to play crucial roles in voluntary motor control. Note that thalamus (blue) is the node through which the basal ganglia and cerebellum both communicate with cortex, and through which the cerebellum communicates with the basal ganglia (cortical-basal ganglia-cerebellar loops).

Krakauer 2000, Hikosaka, Nakamura et al. 2002). I realize that this is generally understood by someone studying motor control in mammals, but I want to make it explicit because much of the Discussion focuses on the extent to which thalamus in songbirds resembles motor thalamus in mammals. As I will review below, a thalamic nucleus in the song system is modulated by basal ganglia output and drives a cortical region that in turn biases the motor cortex-like regions required to execute vocalization. Note that the very simplified description of mammalian motor systems that I have just given includes motor thalamus but not the intralaminar thalamic nuclei, the main source of thalamic input to striatum. Note also that this admittedly oversimplified description of motor systems does not take into account how the corticospinal projections (Dum and Strick 1996) interact with other descending motor pathways such as the rubrospinal system that originates in the red nucleus (Kuypers 1981, Holstege 1991), even though cerebral cortex and the cerebellum both interact with the red nucleus and the output of all three regions ultimately influences the activity of motor neurons. Again as I will review in songbirds there are descending motor pathways that involve a motor cortex-like region, the red nucleus, and the cerebellum, although these pathways are not typically thought to interact with the song system.

Of course when a specific network of brain areas controlling some type of movement is considered, the general description of motor systems I have just given begins to break down. Take for example control of the eye. If what is known about eye control is mapped onto this general description, then the frontal eye fields (Schnyder, Reisine et al. 1985) in cortex would be the central node controlling movement. Yet years of research have established that the cerebellum forms a “side loop” that calibrates eye movements (Optican and Robinson 1980) and that it does so through disynaptic and

trisynaptic routes through the brain stem that don't involve motor cortex; the established models for the cerebellar contribution to eye movement usually neglect the contributions of motor cortex (Manto, Bower et al. 2012). Similarly a large body of work has focused on the role the basal ganglia might play in controlling saccades via its output from part of the substantia nigra to the superior colliculus. Some have argued that the basal ganglia can also directly modulate brainstem nuclei through its output as a result of multiple parallel loops that do not require participation of cortex (McHaffie, Stanford et al. 2005). So I recognize that even within mammals there may be important differences between how motor systems control different movements. In spite of this I will adopt the framework summarized in Figure 1 to compare mammalian motor systems to the song system, for two reasons. First, most readers will be familiar with it and I hope it will make the Introduction and Discussion more readable and understandable to all. Second, at least one attempt has been made to map the predominant model for the function of the songbird basal ganglia onto motor control of saccades in primates (Fee 2012) where there is already a well-developed literature on the functions of the basal ganglia and the cerebellum. These proposed models of oculomotor learning make specific predictions about the roles of thalamostriatal projections. In the Discussion I address the question of how such predictions can be integrated into that model of the songbird basal ganglia function.

I.2.A.2 The song system in songbirds

Having given an overview motor systems in mammals, I give a similar overview the song system in the songbird brain. The discovery that the songbird brain contains a network of discrete nuclei that control song (Nottebohm, Stokes et al. 1976) made possible many studies investigating the

neural basis of learned vocalizations. These vocal control nuclei, now known as the song system (Figure 2), provide a tractable model for dissociating the neural basis of learning and producing vocalizations.

Once song is learned, its production depends on a descending pathway in the song system that begins with nucleus HVC and continues to one of its two main targets, RA (letters HVC now used as a proper name, see (Reiner, Perkel et al. 2004) for discussion of nomenclature; RA stands for Robust Nucleus of the Arcopallium). Lesions of HVC and RA severely impair and in some cases abolished song production (Nottebohm, Stokes et al. 1976). RA projects directly to motor neurons in the hypoglossal nucleus of the brainstem (nXIIts), i.e., the motor neurons that innervate the muscles of the syrinx, the vocal motor organ in birds. Thus RA can be thought of as analogous to motor cortex. Because both HVC and RA are required to produce vocalizations, they form what is often called the vocal motor

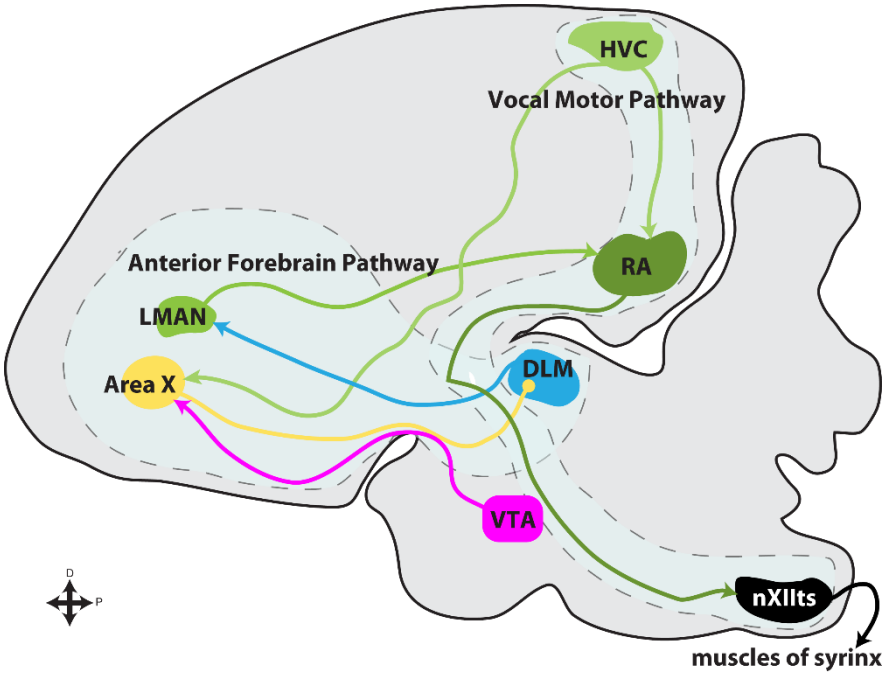

Figure 2. The Song System There are two main pathways in the song system, the vocal motor pathway (VMP) that is required to produce song, and the anterior forebrain pathway (AFP) that is required for learning song. The AFP is of particular importance to the studies in this dissertation. It consists of a thalamocortical-basal ganglia loop, where Area X is the basal ganglia nucleus of the song system, DLM is the thalamic nucleus, and LMAN is a cortical area whose projections to RA constitute the output of the AFP.

pathway (VMP). The other principal target of HVC is Area X. Later studies showed that Area X was part of a separate pathway that, although it was not required for producing song, was required for learning song during development (Bottjer, Miesner et al. 1984) and for inducing plasticity in adult song. Area X is the basal ganglia nucleus of the song system, and the pathway it sits within is known as the Anterior Forebrain Pathway (AFP).

I.2.A.3 The basal ganglia in mammals and songbirds

Both of the unanswered questions about neuroanatomy addressed by this dissertation concern the AFP. For that reason, before I review the literature with respect to these unanswered questions, I first provide background on the current understanding of basal ganglia function in mammals and in the song system of songbirds.

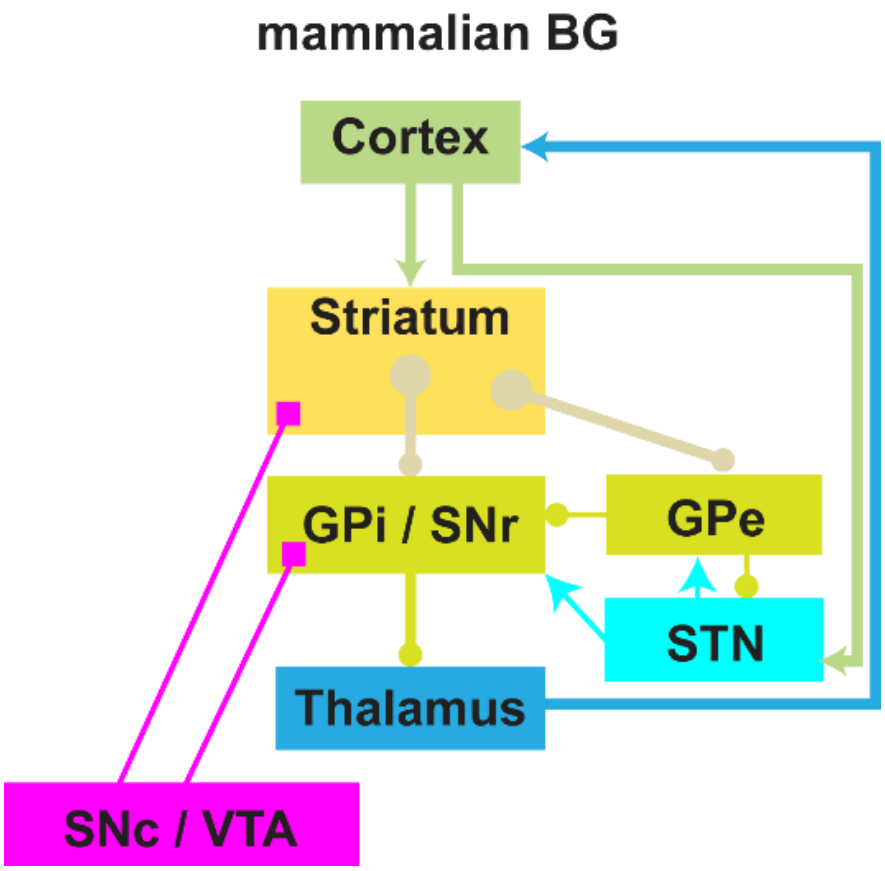

Figure 3. The mammalian basal ganglia. See text for description.

I.2.A.3.a Mammalian basal ganglia anatomy

I begin by outlining the anatomy of the mammalian basal ganglia, as depicted in Figure 3, so that I can later draw comparisons between it and the AFP. The input nucleus of the basal ganglia, the striatum (pale yellow box in Figure 3), receives afferents from cortex (pale green box). In the striatum the principal cell type is the medium spiny neuron (MSN, depicted as beige "neurons" in Figure 3), making up 90-95% of all striatal neurons (Kemp and Powell 1971). MSNs integrate several types of input: excitatory input from cortex; dopaminergic input from the substantia nigra pars compacta and ventral tegmental area of the midbrain (magenta box); and inhibitory input from three GABAergic interneurons and modulatory input from acetylcholinergic interneurons (Kawaguchi, Wilson et al. 1995) (not pictured in Figure 3 to maintain readability). In addition, MSNs also receive excitatory input from thalamus, as do paravalbumin expressing GABAergic interneurons, and the acetylcholinergic neurons. These projections from thalamus to striatum, known as thalamostriatal system, have not been integrated into models of basal ganglia function (DeLong and Wichmann 2009), and so are also not pictured in the diagram. Because one of the questions this dissertation addresses is whether there are thalamostriatal projections in the song system, I discuss this system in mammals in detail in section I.2.A.4. There are two pathways out of the striatum, both through MSNs. The "direct" pathway consists of the population of MSNs that projects to the internal globus pallidus and substantia nigra reticulata (GPi/SNr, bright green box). Note this projection is inhibitory (inhibitory projections indicated with circle in Figure 3). The projection from GPi/SNr to the thalamus is also inhibitory. Indirect pathway MSNs project to the external globus pallidus (GPe, bright green box) that projects to the subthalamic nucleus (STN,

pale blue box). STN projects to GPi/SNr, completing the indirect pathway. Hence GPi/SNr integrates output from the direct and indirect pathways that it communicates to thalamus, and thalamus projects back onto cortex. In addition to the direct and indirect pathways, a hyperdirect pathway is recognized, consisting of the projections from cortex to STN, providing a route for cortex to influence thalamus that bypasses the striatum.

The anatomical studies whose results I have just summarized gave rise to the idea that there is a canonical set of connections that interconnect the basal ganglia and cortex, known as thalamocortical-basal ganglia loop. More detailed anatomical studies revealed that the basal ganglia in mammals consist of multiple parallel loops, each with a distinct function: a skeletomotor loop, oculomotor loop, limbic loop, and so on (Alexander, DeLong et al. 1986). While the loops were seen as largely segregated, each consisted of the canonical set of connections between cortex, thalamus, and the basal ganglia nuclei. There is evidence that the loops are interconnected, in that output from one loop can project to other cortical regions besides the region that provides input to that loop (Joel and Weiner 1994).

I.2.A.3.b Songbird basal ganglia anatomy

I now describe the AFP in detail. As noted above, the AFP includes Area X, the basal ganglia nucleus of the song system. It also includes two other nuclei: DLM, a thalamic nucleus that receives the output of Area X, and LMAN, a cortical nucleus that sends output to RA and also sends collaterals to Area X (Figure 2). As this set of connections implies, the AFP is thus a thalamocortical-basal ganglia loop within the song system. Area X sits within the avian basal

ganglia, specifically within the medial striatum. Its major inputs are the aforementioned projection from cortical area HVC, and a massive dopaminergic projection from the ventral tegmental area (VTA) (Lewis, Ryan et al. 1981, Bottjer, Halsema et al. 1989, Person, Gale et al. 2008). The output neurons of Area X project to thalamic nucleus DLM (medial nucleus of dorsolateral thalamus). The input that DLM receives from Area X is GABAergic and inhibitory (Luo and Perkel 1999, Luo and Perkel 1999), as is basal ganglia input to thalamus in mammals. DLM in turn projects upon LMAN (lateral magnocellular nucleus of the nidopallium), a cortical nucleus that is the output of the AFP, projecting upon RA (Nottebohm, Paton et al. 1982, Bottjer, Halsema et al. 1989). LMAN also projects back to Area X. Thus the AFP forms a thalamocortical-basal ganglia loop, like the functionally separate but parallel loops in the mammalian brain (Alexander, DeLong et al. 1986). Similarly, it was shown that there were topographically-organized loops within the AFP (Luo, Ding et al. 2001).

While the connections of the AFP are broadly speaking similar to thalamocortical-basal ganglia loops in the mammalian brain, there are important differences. I will explain them in relation to the mammalian basal ganglia. Before focusing on the AFP, I reiterate that half a century of studies have shown that the avian brain contains basal ganglia homologous to those structures in mammals (Reiner, Medina et al. 1998, Reiner, Perkel et al. 2004). This area is densely innervated by midbrain dopaminergic neurons. It contains a striatum that is the target of cortical neurons. I also emphasize that the lateral part of the avian striatum (Figure 4), like mammalian striatum, receives dopaminergic input from the substantia nigra pars compacta and projects to a globus pallidus (GP).

GP projects to a thalamic nucleus, DIP, and is reciprocally connected with the subthalamic nucleus (STN) (known as Ansa Lenticularis before changes in nomenclature to better reflect homology) (Jiao, Medina et al. 2000, Person, Gale et al. 2008). The medial striatum is less similar to the well-studied pathways in the mammalian basal ganglia. In songbirds, it contains Area X, the basal ganglia nucleus of the song system. Anatomical and physiological studies have shown that Area X (and the medial striatum) has both striatal and pallidal features (Carrillo and Doupe 2004, Reiner, Laverghetta et al. 2004), as shown in Figure 5. This view of Area X as "striatopallidal" is supported by a wealth of immunohistochemical (Gale and Perkel 2010) and electrophysiological (Farries and Perkel 2002, Goldberg, Adler et al. 2010, Goldberg and Fee 2010) data. All four cell types in the mammalian striatum identified by their intrinsic properties can also be identified in whole-cell recordings of Area X made from brain slices: the sparsely-firing medium spiny neurons, the fast-spiking interneurons, the tonically active neurons (presumed to be the cholinergic interneurons), and the low threshold spiking interneurons (Farries and Perkel 2002). Immunohistochemical studies confirm that these cells include the abundant

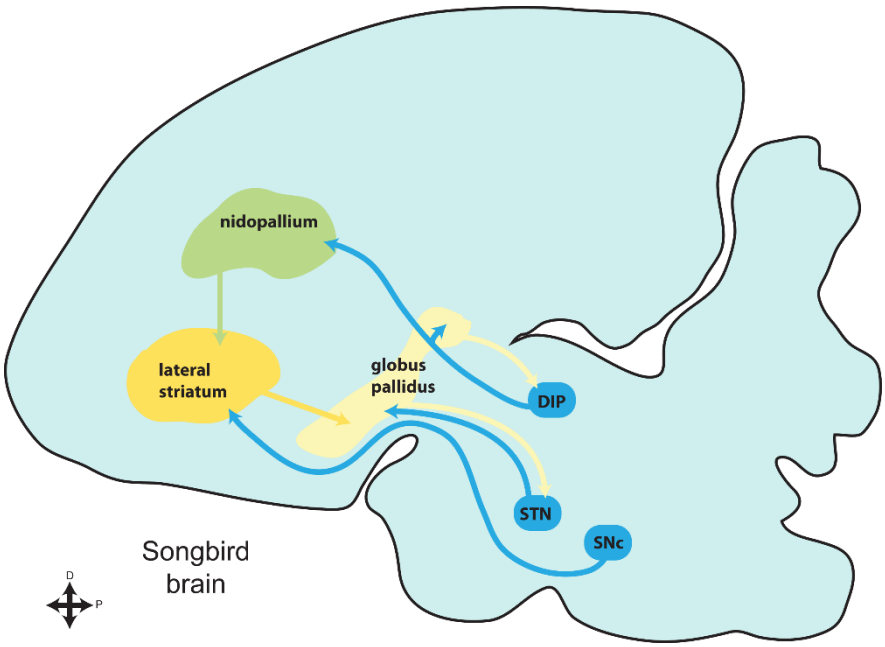

Figure 4. Lateral basal ganglia in the songbird. Description in text.

medium spiny neurons that in mammals are the output of the striatum, as well as the cholinergic and paravalbumin interneurons (Carrillo and Doupe 2004, Reiner, Laverghetta et al. 2004). As in the mammalian basal ganglia, the medium spiny neurons in Area X are the target of glutamatergic input from cortical regions (Farries, Ding et al. 2005): specifically, Area X receives input from cortical nuclei of the song system LMAN and HVC. Extracellular recordings in singing birds also demonstrate that neurons in Area X have firing patterns similar to the four types found in the mammalian striatum (Goldberg and Fee 2010).

In addition to these neurons with striatal-like firing patterns, Area X contains neurons whose firing patterns during song resemble activity of pallidal neurons in the mammalian basal ganglia (Goldberg, Adler et al. 2010). In whole-cell recordings from brain slices, these neurons also have intrinsic properties resembling those of mammalian pallidal neurons (Farries and Perkel 2002). The pallidal-like cells are the sole source of output from Area X. They project to thalamic song system nucleus DLM where they form one-to-one specialized calyceal synapses with thalamic neurons. The specialized output of Area X is not unique to songbirds and is shared with the surrounding medial striatum in the avian basal ganglia (Farries, Meitzen et al. 2005).

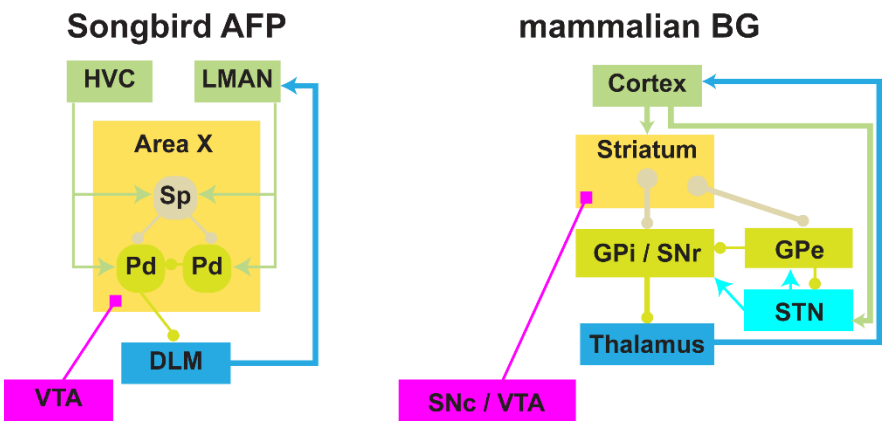

Figure 5. Comparison of the songbird Anterior Forebrain Pathway (AFP) with mammalian thalamocortical-basal ganglia pathway. Description in text. Adapted from (Gale and Perkel 2010).

These findings support the hypothesis that Area X and the surrounding medial striatum combine striatal and pallidal features in one brain area. How similar is the function of Area X to the mammalian basal ganglia if the structures differ? Consider the principal routes in the mammalian basal ganglia, the direct and indirect pathways (Figure 5). The direct pathway begins with MSNs that project to the internal globus pallidus (GPi) and the SNc, both of which project to thalamus. Direct pathway MSNs mainly express D1-type dopamine receptors and contain Substance P. In contrast, the indirect pathway begins with MSNs that project to the external globus pallidus (GPe) that then projects to GPi. MSNs in the direct pathway mainly express D2 receptors and are positive for encephalin. There are no obviously homologous pathways through Area X, but there do appear to be functionally similar pathways. Unlike mammalian MSNs, those in Area X express a mix of D1 and D2 receptors (Kubikova, Wada et al. 2010). They also appear to express Substance P (Carrillo and Doupe 2004), and substance P-positive fibers closely appose the neurites of the pallidal-like output neurons (Reiner, Laverghetta et al. 2004). (Evidence for encephalin in Area X is less clear (Carrillo and Doupe 2004, Reiner, Laverghetta et al. 2004))While there is no globus pallidus-like region between Area X and thalamus, there do appear to be two distinct populations of pallidal-like neurons in Area X. One population projects to DLM and so can be labeled by tracer injections in DLM (Reiner, Laverghetta et al. 2004, Farries, Ding et al. 2005). A separate population is not retrogradely labeled by injections in thalamus, but like the first shows immunoreactivity for the neuropeptide LANT6 and has the same morphology of large soma and distinctive beaded neurites (Reiner, Laverghetta et al. 2004). Slice physiology also demonstrated that the population that is not retrogradely labeled by tracer injections by DLM does have GP-like membrane properties(Farries, Ding et al. 2005). Crucially these experiments showed that axons of

the unlabeled population closely appose dendrites of the population that projects to DLM (Farries, Ding et al. 2005), i.e., they do not represent a direct pathway from Area X to DLM, but indirectly control Area X output. Extracellular recordings from singing birds also support the idea that the local interneurons with pallidal properties behave similarly to GPe neurons in the mammalian basal ganglia while pallidial-like neurons that project to DLM behave similarly to mammalian GPi neurons (Goldberg, Adler et al. 2010). So although there is no evidence for distinct direct and indirect pathway MSNs in Area X, there is evidence that a population of GPe-like neurons plays a role functionally similar to that of the indirect pathway in the mammalian basal ganglia.

I.2.A.3.c Basal ganglia output to motor thalamus

In this subsection I focus on studies of how basal ganglia output influences the activity of neurons in motor thalamus. These studies are relevant to this dissertation for two reasons. First of all, any model of the function of the basal ganglia and what it communicates to cortex via thalamus must account for the results from these studies. Second, basal ganglia output to thalamus also influences activity of thalamic projections back to the striatum. Neurons in basal ganglia-recipient regions of motor thalamus and the intralaminar nuclei have both been shown to send out axon collaterals that terminate in striatum. As noted in the previous section, current models of basal ganglia function do not include a role for the thalamostriatal system. By extension these models have not considered how feedback provided by a reciprocal connection from thalamus would affect basal ganglia activity. The question of how the inhibitory output of the basal ganglia regulates thalamic neuron activity has also been a major focus in studies of the song system. As I show in the results, there

is a projection from thalamus to the basal ganglia in the Anterior Forebrain Pathway, and this result should be integrated into current models of song system function.

Anatomical and physiological studies make it clear that basal ganglia input can have a strong influence on neurons in motor thalamus, but it is not clear at all from other studies how this influence plays out during behavior. Results from ultrastructural studies show that in ventromedial (motor) thalamus of rats, axons from substantia nigra pars reticulata form giant terminals on large diameter dendrites and soma, with each terminal containing multiple synapses (Bodor, Giber et al. 2008). Intracellular recordings from motor thalamic neurons report large amplitude inhibitory post synaptic potentials, with a short latency consistent with a monosynaptic pathway, after stimulation in the globus pallidus or substantia nigra pars reticulata (Chevalier and Deniau 1982, Ueki 1983). This anatomical and physiological evidence led to one proposal of how the basal ganglia might modulate motor thalamic activity: rebound spiking. It was thought that the basal ganglia might maintain thalamic neurons in a hyperpolarized state so that upon rebound of the membrane potential the neurons would emit bursts (mediated by ion channels found in thalamic neurons that are deinactivated by hyperpolarization (Jahnsen and Llinas 1984)). However in mammals there is virtually no evidence that such rebound spiking occurs during behavior (Bosch-Bouju, Hyland et al. 2013). Slice experiments similarly find that the basal ganglia inhibit motor thalamus but do not reliably elicit rebound spikes in mammals (Edgerton and Jaeger 2014).

Not only is there little evidence for rebound spikes, there is also little evidence that the output of the basal ganglia influences activity in motor thalamus in a way that is different from the output of the cerebellum (cerebellar projections to thalamus are excitatory). In some extracellular

recordings made during behavioral experiments, neurons in the basal ganglia and cerebellar-recipient zones of motor thalamus were identified by microstimulation, and the microstimulation in the output nuclei of the basal ganglia strongly inhibited motor thalamic neurons (Anderson and Turner 1991, Nambu, Yoshida et al. 1991). Surprisingly, however, these studies do not find any obvious difference between activity of motor thalamic neurons that receive basal ganglia output and those that receive cerebellar output.

These findings—or lack thereof—suggest that it is probably not the case that basal ganglia and cerebellar output drive motor thalamic activity in the same way that ascending input from the periphery drives neural activity in the sensory thalamic nuclei (Bosch-Bouju, Hyland et al. 2013). Although basal ganglia and cerebellar inputs to motor thalamic neurons are relatively strong, like the ascending inputs that drive sensory thalamic neurons, the motor thalamic neurons also receive projections from cortex as well as other subcortical regions. Some authors have suggested that cortical inputs "drive" motor thalamic activity while basal ganglia output only modulates it (Anderson and Turner 1991, Goldberg, Farries et al. 2012), but it is unclear to what extent cortical input drives motor thalamus (Galvan, Hu et al. 2016). Ultimately it will be necessary to record during behavior simultaneously from neurons in an output nucleus of the basal ganglia, e.g. globus pallidus, and the targets of those neurons in thalamus to demonstrate how basal ganglia output shapes spiking patterns in motor thalamus. Obviously this would be technically challenging. Because of specializations in the song system, similar studies are tractable in songbirds, as I describe in the next section.

I.2.A.3.d Basal ganglia output to thalamus in the AFP

As with mammals, many studies in songbirds have investigated whether in songbirds the output of pallidal-like neurons in Area X controls firing of thalamic song system nucleus DLM through a disinhibitory mechanism. The specialized one-to-one calyceal axon terminals that Area X striatopallidal neurons form in DLM (Luo and Perkel 1999, Luo and Perkel 1999) suggest the inhibitory output of the basal ganglia is particularly important within the AFP. Studies in slice (Person and Perkel 2005) and in anesthetized birds (Leblois, Bodor et al. 2009) suggested that Area X might trigger spikes through a post-inhibitory rebound mechanism. As I outlined above, though, the few studies carried out in mammals found that globus pallidus increases its firing before movements and that pallidal-recipient motor thalamus increases its firing rate at the same time. Those studies concluded that excitatory drive from cortex might have more of an effect on motor thalamus than pallidal input. Because of the specialized calyceal synapse in DLM from Area X, it is possible to record Area X input to a DLM neuron and the spiking of that DLM neuron simultaneously. Goldberg and Fee (2012) took advantage of this to record thalamic and pallidal activity in singing birds. Their results were similar to what was found in mammals. As they had shown previously, thalamic neurons often had a peak in firing rate just before syllable onset and a decrease at syllable offset. Unexpectedly, pallidal inputs also showed peaks at onsets and dips at offsets, and hence were not permitting DLM neurons to fire via disinhibition. Area X inputs to DLM did completely inhibit firing but this inhibition lasted only on the order of 5-10 ms. Goldberg and Fee further provided evidence that a driver of firing in DLM is in fact input from RA, i.e. the cortical input to motor thalamus in the song system. Peaks in DLM firing rate at syllable onset

persisted even after lesions of Area X. Regarding the Goldberg-Fee model described above, it cannot then be the case that the output of Area X to LMAN through DLM is conveyed by disinhibition. In discussing their results, Goldberg and Fee observe that the strong inhibitory effects of Area X input to DLM allows for fine temporal control of spiking. As an alternative hypothesis, they propose that spike-timing dependent plasticity would provide another mechanism for Area X to convey computed reward to LMAN via DLM, instead of disinhibition. With respect to their results, it is also surprising that RA should be such a potent driver of DLM given that the original report of this projection (Vates, Vicario et al. 1997) seems to show that RA mainly targets another thalamic song system nucleus DMP, sending relatively sparse targets to DLM. No study has systemically investigated the relative strengths of the projection—anatomically or physiologically—from RA to different regions of thalamus. I am not aware of any studies in mammals that similarly record pallidal and thalamic activity simultaneously during movement, although the question of how they interact has been identified as crucial to understanding basal ganglia function (Nambu 2008). In mammals as in songbirds thalamic neurons do exhibit rebound spiking (Jahnsen and Llinas 1984) that could potentially be elicited by strong inhibition, and the pallidal input to thalamus does take the form of "driver"-like terminals (Bodor, Giber et al. 2008) similar to the specialized calyx-like terminal observed in songbirds. ("Driver" and "modulator" inputs are terms used in one proposed schema for characterizing post-synaptic effects of neurons (Sherman and Guillery 1998).) A slice study in mice did show that basal ganglia output to thalamus in mammals is more likely to regulate firing through classic inhibition than by eliciting rebound spikes (Edgerton and Jaeger 2014). But as referenced above a study of the effects of the projection from cortex to motor thalamus (Galvan, Hu et al. 2016) found that this projection was not a main

driver of activity but rather a modulator, leaving open the question of how the pallidal and cortical inputs interact during movement.

Across mammals and birds, the findings from different experimental approaches seem to offer a paradoxical view of how basal ganglia output regulates the thalamus (Goldberg, Farries et al. 2013). One model has been proposed for how thalamic neurons integrate output from the basal ganglia that explains all the different findings (Goldberg, Farries et al. 2012), but it seems likely that more experiments in behaving animals will be needed to definitively address this question.

I.2.A.3.e Basal ganglia function and theoretical models

Given the anatomy of the basal ganglia described above, and the effects of its output on motor thalamus reviewed in the previous section, what can we conclude about the function of the basal ganglia? I briefly review one model of basal ganglia function, the action selection model. This is a circuit level model, by which I mean that it includes the connections between areas just described, and it accounted for most of the physiological results I have just summarized. However, behavioral experiments have undermined support for the action selection model. A significant body of evidence now points towards two other proposed functions of the basal ganglia: learning motor skills and controlling vigor. The term vigor refers to parameters of movement such as speed and force, and is meant to connote motivation—I expand this definition below. To my knowledge, there are no circuit level models like the action selection model that are broadly accepted as a basis for further investigations of learning motor skills and controlling vigor in mammals. The evidence for these functions comes in part from songbird studies, and there is a circuit level model of the

AFP in songbirds, built upon a reinforcement learning framework. I describe this model so that in the Discussion I can integrate my anatomical results into the model, make predictions about function, and propose follow-up experiments.

The core idea of the action selection model (Penney and Young 1983) is that disinhibition of thalamus by striatum inhibiting globus pallidus would allow selection of one action while others would be inhibited. The model sought to explain normal function of the basal ganglia and at the same time explain symptoms seen in movement disorders (Albin, Young et al. 1989, Delong 1990). Thus the action selection model predicted that slowed movement initiation in Parkinson's arose due to increased activity in the indirect pathway that yielded increased inhibition of the motor thalamus, and that the chorea (sudden movements) seen in Huntington's disease result from striatal degeneration and an ensuing disinhibition of motor thalamus. Later updates to the model re-framed it as "focusing" where the hyperdirect pathway from cortex through the subthalamic nucleus to thalamus could focus the selected behavior while globus pallidus would inhibit other competing behaviors (Mink 1996).A principal complaint against the action selection model is that, based on the number of synapses in each pathway, one would *a priori* expect that actions could be selected by the direct pathway before other actions are inhibited by the indirect pathway (DeLong and Wichmann 2009). A dynamic model (Nambu 2004) took these transmission times into account and thus does make specific predictions about how basal ganglia output would influence neuronal activity in motor thalamus. It posits that initial excitation of GPi/SNc via the hyperdirect pathway would inhibit motor thalamus, but then inhibition of GPi/SNc via the direct pathway would allow motor thalamus activity to increase, and finally renewed excitation of GPi/SNc via the indirect

pathway would again inhibit motor thalamus. Unfortunately, this model does not agree with findings from single unit recordings of GPi and motor thalamus neurons in hand movements that showed that activity in both regions increases at the same time, at least at a population level (Anderson and Turner 1991).

However, experimental evidence does provide support for two other proposed functions of the basal ganglia: learning motor skills (Shmuelof and Krakauer 2011, Graybiel and Grafton 2015) and controlling vigor (Mazzoni, Hristova et al. 2007, Shadmehr and Krakauer 2008, Turner and Desmurget 2010). I begin with the evidence that the basal ganglia are required for acquisition of motor skills. Studies in mammals show that sequential tasks cannot be learned (Shmuelof and Krakauer 2011) after lesions (Eckart, Huelse-Matia et al. 2010, Moussa, Poucet et al. 2011) or during pharmacological activation of the basal ganglia (Miyachi, Hikosaka et al. 1997). Similarly, it is known that the AFP is required for songbirds to learn their song as juveniles (Bottjer, Miesner et al. 1984, Sohrabji, Nordeen et al. 1990). Furthermore, after a motor skill is learned, the basal ganglia are not required to execute that skill as shown in mammals (Desmurget and Turner 2010) and songbirds (Bottjer, Miesner et al. 1984, Nordeen and Nordeen 1993). Therefore the basal ganglia specifically contribute to learning a skill, but implicit memories of the skill are stored elsewhere, presumably cortex (although one study found that a learned motor skill was not stored in cortex in rats (Kawai, Markman et al. 2015). Whether this finding holds may depend on the definition of a learned motor skill, and this definition could change as we better understand what types of movements are under control of different brain regions, which may vary across species (Dudman and Krakauer 2016)).

Although there is good evidence the basal ganglia are required for learning a motor skill, there is less evidence for *what* they contribute. And of course it is possible that the basal ganglia could be required for learning, and *also* contribute to movement after learning. I first review evidence from mammals that the basal ganglia contribute vigor to movement. Then I turn to results from studies of songbirds that support the idea that the AFP contributes similarly to song.

There is also good evidence from studies of mammals and songbirds that the basal ganglia contribute vigor to movement (Mazzoni, Hristova et al. 2007, Turner and Desmurget 2010, Dudman and Krakauer 2016). The name "movement vigor" was chosen by analogy with the term "response vigor" used in studies of extrinsic reward mediated by the nucleus accumbens and other limbic regions in the basal ganglia. The idea behind the name is that motor-related regions of the basal ganglia carry out a similar cost/benefit calculation that influences the parameters of movement such as speed, velocity, etc. Support for this idea comes from studies of slowed movement in Parkinson's patients (Mazzoni, Hristova et al. 2007) where the term "movement vigor" was first proposed. Similarly, the main effect of pharmacologically inactivating GPi in nonhuman primates was on movement parameters (Kato and Kimura 1992, Desmurget and Turner 2008, Desmurget and Turner 2010). These are the same studies that found GPi was not required to recall sequential movements (Desmurget and Turner 2008, Desmurget and Turner 2010), and in addition they found no effect on reaction time, or on-line correction of movement errors, both of which weaken the case for action selection models. Songbird studies have also provided support for the idea that the basal ganglia compute vigor. Songbirds modify kinematics of song based on social context (Hessler and Doupe 1999, Woolley 2016 and references therein). Output from the

basal ganglia is required for this modulation based on social context, and so most workers view this as a reward-like computation.

Studies of the songbird AFP have provided evidence that the function of this basal ganglia-thalamocortical loop in the song system is to control variability of song and to bias motor output. The terms “variability” and “bias” have specific meanings within songbirds studies that will become clearer as I review the results, but as those terms suggest, the overall results from these studies are consistent with findings from mammals that the basal ganglia contribute vigor to movement.

There are two lines of research within which variability in song has been studied. The first deals with social context. Studies with Zebra finches and Bengalese finches have shown that males reduce the variability of pitches they sing when directing song to a female (Hessler and Doupe 1999, Kao, Doupe et al. 2005, Woolley 2016 and references therein). In comparison, there is more variability in “undirected” song, sung alone. Output from the basal ganglia is required for this modulation based on social context. Specifically, cortical nucleus LMAN in the AFP seems to be the source of variability during different social contexts (Kao and Brainard 2006, Hampton, Sakata et al. 2009), during undirected song (Kao, Doupe et al. 2005), and during development (Aronov, Veit et al. 2011). Interestingly, lesion studies of Area X suggest that this basal ganglia nucleus attenuates variability generated by LMAN (Kojima, Kao et al. 2013). Taken together, these results are consistent with the idea that the AFP performs a reward-like computation similar to movement vigor.

The second line of research studying variability in song asks how it contributes to the ability of the bird to modify song after acquisition, and this line of studies has also shown that the AFP contributes bias to song. Initial evidence that the variability that remains in song after it is learned might be related to plasticity came from studies showing adult birds retain the ability to shift the pitch of their song. This was shown with an operant conditioning paradigm, in which one syllable of a bird's song is targeted with a computer that can deliver an aversive white noise blast. When delivery of the white noise blast is made contingent on whether the syllable is sung above or below some threshold set on the distribution of sung pitches, the bird will shift that distribution to sing less of the punished pitches (Tumer and Brainard 2007). (Results from a typical experiment are shown in Figure 6) Pharmacological inactivation of LMAN shows that it provides a

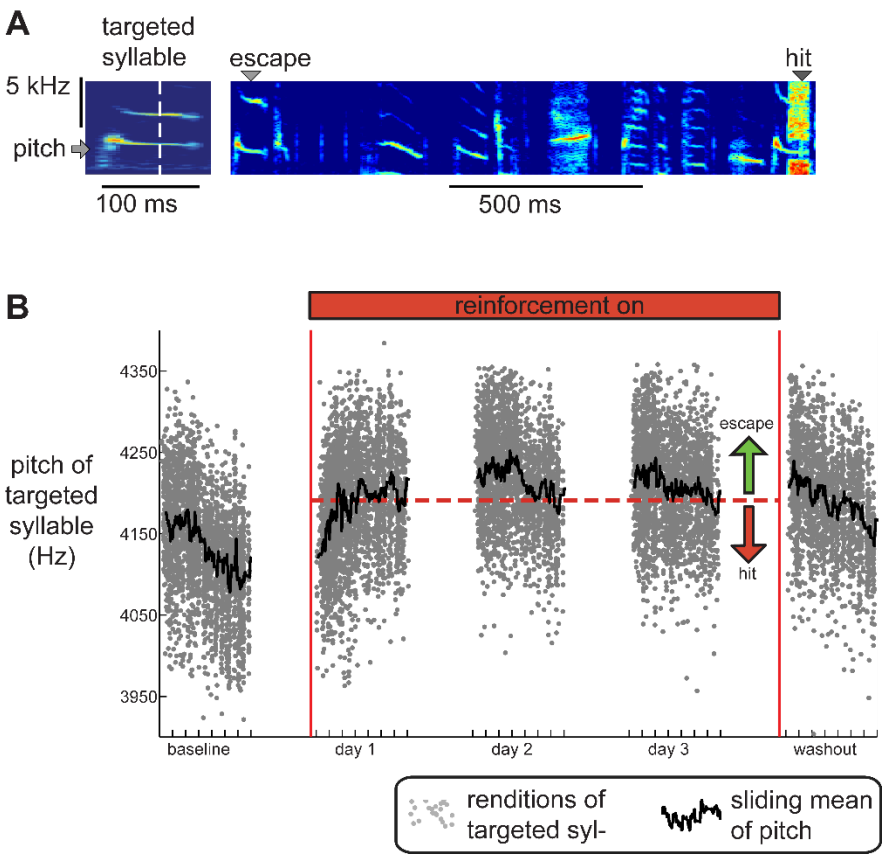

Figure 6. Schematic of aversive reinforcement experiments. **A,** left panel: example of a syllable targeted in a typical experiment. Pitch is estimated from one of the harmonics as described in methods. Right panel, example song bout with two renditions of targeted syllable, one where sung pitch allowed bird to "escape" white noise and another that was a "hit". **B,** plot of pitch of targeted syllable from a typical experiment over three days. One baseline day before reinforcement and a washout day after are shown for comparison.

bias that drives the direction of pitch shift, but that over the course of days the shift to nucleus RA in the motor pathway (Andalman and Fee 2009, Charlesworth, Warren et al. 2012).

In summary, results from studies of mammals and songbirds provide a broad base of support for the idea that the basal ganglia are required for learning motor skills (Shmuelof and Krakauer 2011), and that the basal ganglia contribute vigor to movement when a motor skill is executed (Turner and Desmurget 2010, Dudman and Krakauer 2016). Typically studies in mammals have focused on task level aspects of movements within the context of operant conditioning, and have neglected to measure how the performance of a skill improves across trials (Shmuelof and Krakauer 2011). Studies of songbirds have explicitly examined on a trial-by-trial bias how the basal ganglia contribute variability and bias during execution of a learned motor skill (Shmuelof and Krakauer 2011). However no study in songbirds has provided direct evidence that it is the bias from the AFP that improves song as a juvenile practices during development (Shmuelof and Krakauer 2011). Behavioral studies in mammals do not provide strong support for the action selection model, at least as it would apply to movements in the context of operant conditioning paradigms. However, there is no similar, widely agreed upon, circuit-level model of how the basal ganglia contribute to motor learning in mammals (DeLong and Wichmann 2009). A circuit-level model has been proposed for the thalamocortical-basal ganglia loop in the song system of the songbird (Fee and Goldberg 2011, Fee 2012) that I describe in the next section. It is also the case that current models of basal ganglia function do not include the thalamostriatal system and cerebellar-basal ganglia interactions (DeLong and Wichmann 2009). I review relevant literature on the thalamostriatal system and pathways from the cerebellum to the basal ganglia below (sections I.2.A.4 and I.2.A.5

) and in the Discussion, I consider how both sets of connections might be involved in the function of the songbird AFP (sections IV.2.A and IV.2.B).

I.2.A.3.f Reinforcement learning in the AFP

As mentioned above, most theories about how song is learned have been cast in the framework of reinforcement learning. I briefly summarize one such hypothesis because it integrates many of the findings I just described and because I will refer to it when discussing the functional implications of the results in this dissertation. The Fee-Goldberg hypothesis (Fee and Goldberg 2011) posits that the AFP, the thalamocortical-basal ganglia loop in the song system, instantiates a reinforcement learning algorithm that biases song towards rewarding performances.They propose that MSNs in Area X integrate information about the three things required for reinforcement learning to take place: action, context, and reward. In this model, HVC inputs to MSNs provide information about context. HVC neurons are known to have an "ultasparse" firing pattern where each fires a brief burst of only a few spikes at a specific time in each rendition of a bird's song (Hahnloser, Kozhevnikov et al. 2002, Kozhevnikov and Fee 2007). Theoretical work supports the idea that these sparse bursts provide a suitable code for conveying state, i.e., context in the sense of what time it is in the song (Fiete, Hahnloser et al. 2004). The topography of HVC projections to Area X is also consistent with the idea that HVC conveys state information to MSNs; single HVC axons branch widely throughout Area X (Fortune and Margoliash 1995). LMAN inputs to MSNs would provide information about action. "Action" in this case is the bias (Charlesworth, Warren et al. 2012) and variability (Kao, Doupe et al. 2005) that LMAN injects into RA, the motor cortex-like nucleus in the song system. This is plausible in that LMAN sends collaterals to Area

X whose topography is similar to the topography of LMAN projections to RA (Nixdorf-Bergweiler, Lips et al. 1995, Vates and Nottebohm 1995). The topography is maintained throughout the AFP loop (Luo, Ding et al. 2001), suggesting there are separate channels, perhaps relating to the myotopy in RA (Vicario 1991). Lastly, the reward prediction error-like signal would be provided to MSNs by the dopaminergic input from VTA (Gadagkar, Puzerey et al. 2016, Hoffmann, Saravanan et al. 2016). In the Goldberg-Fee model, it is ultimately DLM, the thalamic nucleus, that conveys changes acquired by MSNs to LMAN so that LMAN in turn biases RA to produce more rewarding song. The model predicts that DLM would drive LMAN to produce rewarding bias by a disinhibition mechanism: MSNs that acquire burst during a specific time in song would inhibit striataopallidal neurons, silencing their inhibitory output to DLM and thus allowing it to excite LMAN at the appropriate time. Physiological studies have shown that Area X output does not control DLM activity through disinhibition, as reviewed above (section I.2.A.3.d). A recent study shows that the main driver of DLM seems to be cortical input from RA, while inhibitory input from Area X appears to regulate spike timing (Goldberg and Fee 2012). This is interesting given my results showing that DLM also projects to Area X (see section III.2.A). In the Discussion, I consider how this reciprocal connection from DLM to Area X might be integrated into the model of the songbird AFP I have just described (see section IV.2.A.6).

I.2.A.4 The thalamostriatal system: anatomy, physiology, and theories about computation

I.2.A.4.a.1 Anatomy: two types of thalamostriatal projection neurons

The thalamus is a crucial node in motor systems. As stated in section I.2.A.1, it is the set of nuclei in ventral thalamus, known as motor thalamus, that receives output from the basal ganglia and cerebellum and then communicates this output through largely separate channels to motor cortical regions. However, there are other regions of thalamus that receive output from the basal ganglia and cerebellum, namely the intralaminar nuclei. As the name implies, these nuclei are found within the internal medullary lamina, a Y-shaped band of fibers that divides up the mammalian thalamus. The intralaminar nuclei are the source of a significant projection to the striatum. In particular, the centre median/parafiscular complex (CM/Pf) is a part of the intralaminar nuclei in mammals that gives rise to topographically organized projections to all of striatum and cortex (Smith and Parent 1986, Berendse and Groenewegen 1990, Smith, Galvan et al. 2014). This anatomical evidence suggests that CM/Pf is distinct from other parts of thalamus that also project to striatum. In fact, the literature currently recognizes two classes of thalamostriatal neurons (Deschenes, Bourassa et al. 1996, Deschenes, Bourassa et al. 1996, Lacey, Bolam et al. 2007, Ellender, Harwood et al. 2013, Alloway, Smith et al. 2014, Smith, Galvan et al. 2014). One is found in CM/Pf and the other is found in thalamic regions outside CM/Pf. If the song system contains thalamostriatal projections, that finding will raise immediate questions about the cell types that give rise to that projection. For that reason I closely review the evidence for the two classes identified in mammalian thalamus.

There are several characteristics that distinguish these two classes: the morphology of the cells, the morphology of their axon terminals within striatum, the locations on striatal dendrite they target, the cell types within the striatum that they target, the physiological properties of each class, and to some extent the afferent projections to CM/Pf and all other thalamic regions outside of it. These differences are schematized in Figure 7.

Most of the evidence for morphological differences comes from single-cell tracing studies, where a neuron is labeled e.g. by juxtacellularly iontophoresing biocytin. These studies find that the perikarya and dendrites of CM/Pf neurons do not have the same morphology as neurons in other parts of thalamus. Whereas neurons in other

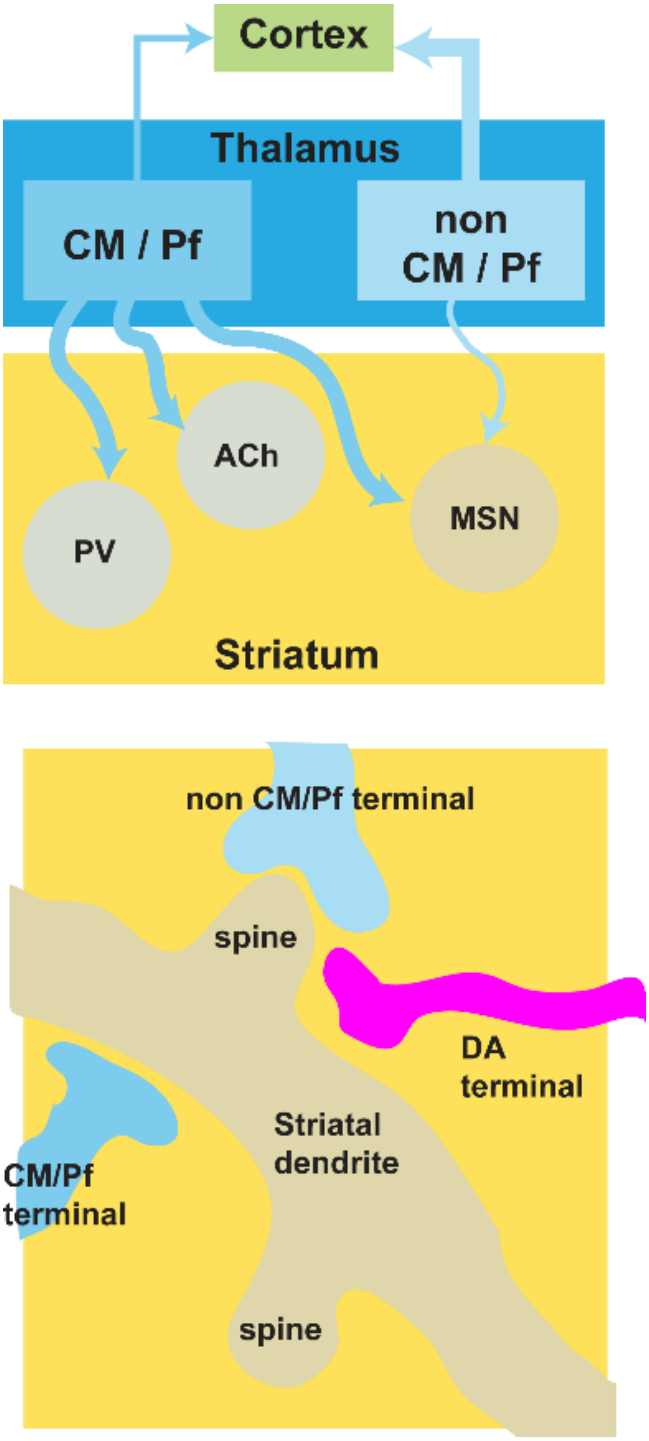

Figure 7. Two types of thalamostriatal projections CM/Pf is the source of a majority of thalamostriatal projections although other thalamic nuclei project sparsely to striatum. The other thalamic regions principally target spines of MSNs, often in conjunction with dopaminergic (DA) terminals. In contrast, CM/Pf terminals target dendrites, not spines, and in addition to MSNs they also target acetylcholinergic (ACh) and parvalbumin-positive (PV) GABAergic interneurons. Adapted from (Smith, Galvan et al. 2014)

intralaminar nuclei have highly-branching "bushy" dendrites typical of thalamic relay neurons, CM/Pf neurons have longer dendrites that branch much less frequently (Deschenes, Bourassa et al. 1996, Parent and Parent 2005, Lacey, Bolam et al. 2007). In addition, the morphology of the axon terminals formed by CM/Pf neurons is distinct from that of other thalamostriatal neurons. Single-axon reconstructions show that CM/Pf neurons send off several collaterals within striatum that can then ramify locally to form a dense cluster of axon terminals (Deschenes, Bourassa et al. 1996, Parent and Parent 2005). In contrast, striatal projections of other parts of thalamus form long branches throughout striatum with varicosities that are *en passant* synapses (Deschenes, Bourassa et al. 1996, Ichinohe, Iwatsuki et al. 2001, Lacey, Bolam et al. 2007). The single-cell tracing studies also show that CM/Pf neurons project only sparsely to cortex with most of the projection being to striatum, while the converse is true for other thalamostriatal neurons (Deschenes, Bourassa et al. 1996, Deschenes, Bourassa et al. 1996, Parent and Parent 2005). Note that one study (Parent and Parent 2005) found diverse cell types in primate Pf: a majority targeted mainly striatum with sparse terminations in cortex, but some targeted only striatum or cortex. Also note that most of the findings on thalamostriatal projections outside CM/Pf are based on studies of CL neurons, another one of the intralaminar nuclei. I am aware of only one study that reconstructed individual thalamostriatal neurons outside the intralaminar nuclei, carried out with viral vectors in rats. Interestingly, that study found that basal ganglia-recipient motor thalamic neurons send out collaterals to striatum, while cerebellar-recipient motor thalamus do not and project only to cortex (Kuramoto, Furuta et al. 2009). Neurons in motor thalamus had “bushy” dendritic trees typical of relay neurons in thalamus but unlike those in CM/Pf. The study focused mainly on cortical projections and did not describe in depth the morphology of axons in striatum. A study of

anterograde tracer injections into primate motor thalamus reported that axon collaterals in striatum included locally dense arborizations with many terminals as well as long segments with varicosities suggestive of *en passant* synapses (McFarland and Haber 2001). Because of the relatively large injections, the authors could not draw conclusions about the morphology of individual thalamostriatal neurons. Overall, single-axon tracing studies support the idea that CM/Pf neurons form a separate class of thalamostriatal neuron.

Evidence for differences in the part of the dendrite targeted by thalamostriatal projections comes from electron microscopy (EM) analysis. Results reveal that CM/Pf terminals in striatum preferentially target dendritic shafts while other thalamostriatal terminals preferentially target dendritic spines (Dube, Smith et al. 1988, Xu, Wilson et al. 1991, Sadikot, Parent et al. 1992, Raju, Shah et al. 2006, Lacey, Bolam et al. 2007). Again there is heterogeneity in the CM/Pf population, though: a study in rats combined EM with single-axon tracing and found that an individual cell might target mainly dendritic shafts but at least one Pf cell almost exclusively targeted dendritic spines (Lacey, Bolam et al. 2007).

Combined immunohistochemical and tracer studies have also revealed a difference between the cell types in striatum targeted by CM/Pf and by other regions of thalamus. Both classes of thalamostriatal neuron target MSNs. Only the CM/Pf neurons, however, have been shown to target acetylcholinergic interneurons (Lapper and Bolam 1992, Sidibe and Smith 1999). In addition, CM/Pf neurons target the somatostatin and parvalbumin containing interneurons found in the striatum (Sidibe and Smith 1999).

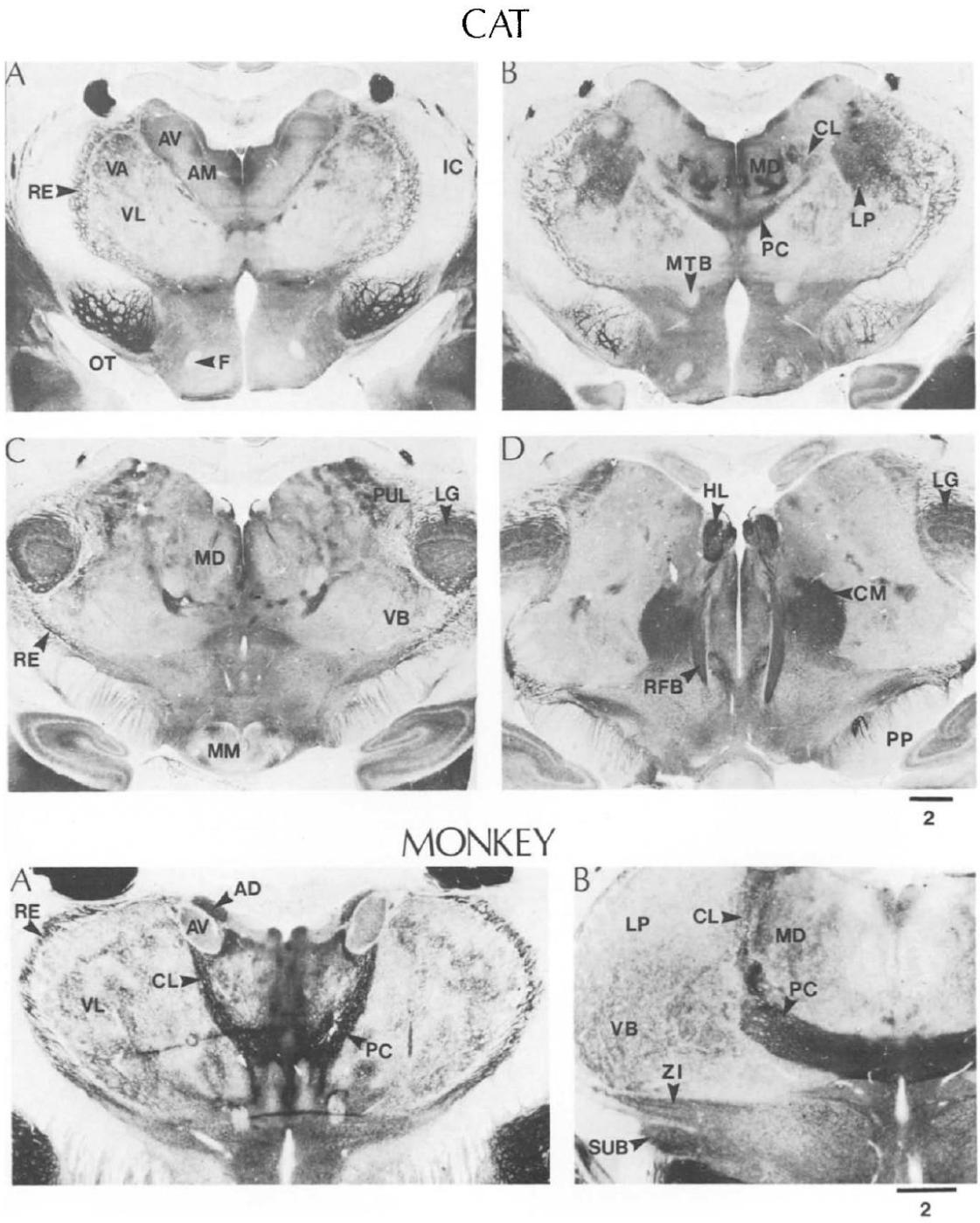

Figure 8. Distribution of acetylcholinesterase activity in the thalamic nuclei in cat and macaque monkey. Staining according to Gomori's technique. Frontal sections. Four levels rostral to caudal (A-D) in cat, and two levels (A and B) in monkey. In this and following figures, horizontal bars indicate mm. Figure 1 from (Steriade, Pare et al. 1988). Used by permission.

I.2.A.4.a.2 Anatomy: afferents of the intralaminar nuclei

Here I review afferents of the intralaminar nuclei, because as I review in section I.2.B, the dorsal thalamus in songbirds that includes thalamic song system nuclei is hypothesized to be the homolog of the intralaminar nuclei. If this hypothesis is true, then afferents of dorsal thalamus in songbirds should be similar to those of the intralaminar nuclei. As I review, little is known about afferents to dorsal thalamus in songbirds (section I.2.B.1.a). In the Discussion (sections IV.2.A.4 and IV.2.A.7), I propose follow-up studies investigating possible afferents to songbird dorsal thalamus, based in part on the literature I review here. In mammals, the afferents to the intralaminar nuclei differ from those of the motor thalamus, supporting the idea that each region of thalamus has a different function in the motor system.

CM/Pf and the other intralaminar nuclei receive major projections from the brainstem reticular formation (Van der Werf, Witter et al. 2002). Stimulation of the brainstem reticular formation and of the intralaminar nuclei can "recruit" cortex, resulting in awake-like brain states in anesthetized animals (Moruzzi and Magoun 1949). Likewise, stimulation of the intralaminar nuclei can have multiple physiological effects in the striatum, suggesting these thalamic afferents serve as an important regulator of striatal activity levels (Smith, Galvan et al. 2014). For these reason the intralaminar nuclei have long been considered part of what is known as the Anterior Reticular Activating System. It should be said that there is evidence that motor thalamic nuclei also receive projections from the same nuclei in the brainstem reticular formation, although retrograde tracer studies suggest this projection is much smaller. This holds true in rats (Newman and Ginsberg 1993), and in cats and monkeys where retrograde label in the brainstem was greater than three

times higher when injecting in the intralaminar nuclei than in other thalamic nuclei (Pare, Smith et al. 1988, Steriade, Pare et al. 1988). Acetylcholinergic cell groups in the brainstem contribute significantly to reticular formation input to the intralaminar nuclei (Hallanger, Levey et al. 1987, Pare, Smith et al. 1988, Steriade, Pare et al. 1988). In fact, histological stains for acetylcholinesterase have been used to identify the intralaminar nuclei (examples are shown in Figure 8, I return to this figure in I.2.A.5.a.1 when discussing different regions of thalamus that receive cerebellar output in mamals.) Serotoninergic cell groups also contribute (Vertes and Martin 1988, Lavoie and Parent 1991, Vertes 1991, Vertes, Fortin et al. 1999) as well as regions of the reticular formation outside of the monoaminergic cell groups (Vertes and Martin 1988, Lavoie and Parent 1994) that seem to be involved more directly with movement.

Another set of afferents to CM/Pf that may distinguish it from the motor thalamus is from the superior colliculus (SC). CM/Pf and another intralaminar nucleus, CL, both show heavy anterograde label after injections of tracer in the deep layers of SC. This part of SC shows multisensory responses to behaviorally-relevant stimuli and has been proposed as another driver of activity in the intralaminar nuclei, along with the brainstem reticular formation (Minamimoto, Hori et al. 2009, Smith, Galvan et al. 2014). Note that CL in addition to SC input receives input from the cerebellum, while CM receives input from both SC and the basal ganglia (Van der Werf, Witter et al. 2002), which is of particular relevance to the studies here. In section I.2.A.5.a.1 I explain in more detail the differences between cerebellar-recipient regions of the intralaminar nuclei and motor thalamus in mammals, as they pertain to the studies in this dissertation.

I.2.A.4.a.3 Physiology and function of the thalamostriatal system

Here I review functions proposed for the two classes of thalamostriatal projections: those originating in CM/Pf and those from other parts of thalamus. I begin with CM/Pf because there is a significant body of literature on the function of this region of the intralaminar nuclei. The literature focuses on the role of CM/Pf in operant conditioning paradigms—I am not aware of any studies that investigated the role of CM/Pf in learning and producing motor skills. Two functions have been proposed for CM/Pf projections to striatum, and they are relevant to this dissertation because both can be understood within a reinforcement learning framework, and as reviewed above, this framework underlies current models of the function of the songbird AFP.

The first function that has been proposed for CM/Pf is to compute "state prediction error" (Bradfield, Hart et al. 2013), by analogy with the reward prediction error that the midbrain dopaminergic cells are supposed to calculate (Schultz, Dayan et al. 1997). A set of studies in rats found that lesions of Pf specifically impair the ability to learn a new mapping from response to outcome (Bradfield, Bertran-Gonzalez et al. 2013), building on previous work (Bradfield, Hart et al. 2013). Pf lesions did not impair the ability to learn that a given response to a stimulus will produce a rewarding outcome. The authors conclude that thalamostriatal neurons activate the striatum when a state does not match the previously predicted state, facilitating reward-related changes in synaptic strength.

The second proposed function, closely related, is for the CM/Pf to "rebias" an action-selection like process in the basal ganglia when an unexpected stimulus occurs, to prevent selection of an action

that would only have been rewarding if a predicted state had occurred (Minamimoto, Hori et al. 2009). A series of studies in primates found that neurons in both CM and Pf respond to behaviorally-relevant stimuli (Kimura, Minamimoto et al. 2004). These studies seem to suggest that the response depends on the "surprise" associated with the stimuli: CM/Pf neurons often responded to stimuli regardless of whether they were associated with a task, but quickly habituated to repeated presentations of a given stimulus (Matsumoto, Minamimoto et al. 2001). As the authors of these studies discuss, increased activity of CM/Pf neurons in response to "surprising" stimuli is consistent with the idea that it could encode state prediction errors (Yamanaka, Hori et al. 2017).

Regulation of acetylcholinergic interneurons by CM/Pf is probably key to whatever function these projections have, as suggested by physiological studies. Lesions or inactivation of CM/Pf can change cholinergic levels in the striatum, although the effect can depend on experimental conditions (Consolo, Baronio et al. 1996, Zackheim and Abercrombie 2005, Nanda, Galvan et al. 2009). Microstimulation of CM/Pf can have effects on the activity of TANs (tonically-active neurons, putative acetylcholinergic interneurons) as well as PANs (phasically-active neurons, putative MSNs) (Nanda, Galvan et al. 2009). Lesions of PF in rats not only impair the ability to flexibly learn reward-outcome associations as just described, but also give rise to long-term downregulation of the tonic spiking of cholinergic interneurons in the striatum (Bradfield, Bertran-Gonzalez et al. 2013). Pharmacologically inhibiting cholinergic interneurons also similarly impairs learning. In contrast to the encoding of "surprising" stimuli seen in CM/Pf, the TANs in striatum

that are targeted by CM/PF projections only acquire a response to the same stimuli after conditioning (Aosaki, Tsubokawa et al. 1994, Yamanaka, Hori et al. 2017).

An additional function proposed for thalamostriatal projections—not necessarily CM/Pf—is to relay motor efference copies to MSNs. This proposed function comes from a theory paper (Fee 2012) that melds reinforcement learning type models with results from the songbird literature and applies them to a series of studies on basal ganglia involvement with saccade generation in primates. There is evidence for motor-related activity in regions of superior colliculus (McHaffie, Stanford et al. 2005) that project to the intralaminar nuclei (Krout, Loewy et al. 2001). One of those intralaminar nuclei, MD, displays activity during saccades consistent with the idea that it relays corollary discharge (Sommer and Wurtz 2002), i.e. an efference copy of motor commands, and MD could relay that activity to MSNs via its thalamostriatal projections (Royce 1983).

The differences between CM/Pf and other thalamostriatal projections, in terms of their morphology, physiology, and proposed roles in behavior, provide testable hypotheses about possible thalamostriatal projections in the songbird. Results from testing these hypotheses should inform our understanding of the function of thalamostriatal systems. I propose such hypotheses in the Discussion (section IV.2.A).

I.2.A.5 Cerebellum: anatomy, physiology, and theories about computation

One major question this dissertation seeks to answer is whether the cerebellum interacts with the song system in songbirds. The cerebellum is the part of the brain that is perhaps best known for its involvement with movement, and specifically with motor learning. There is also evidence that the

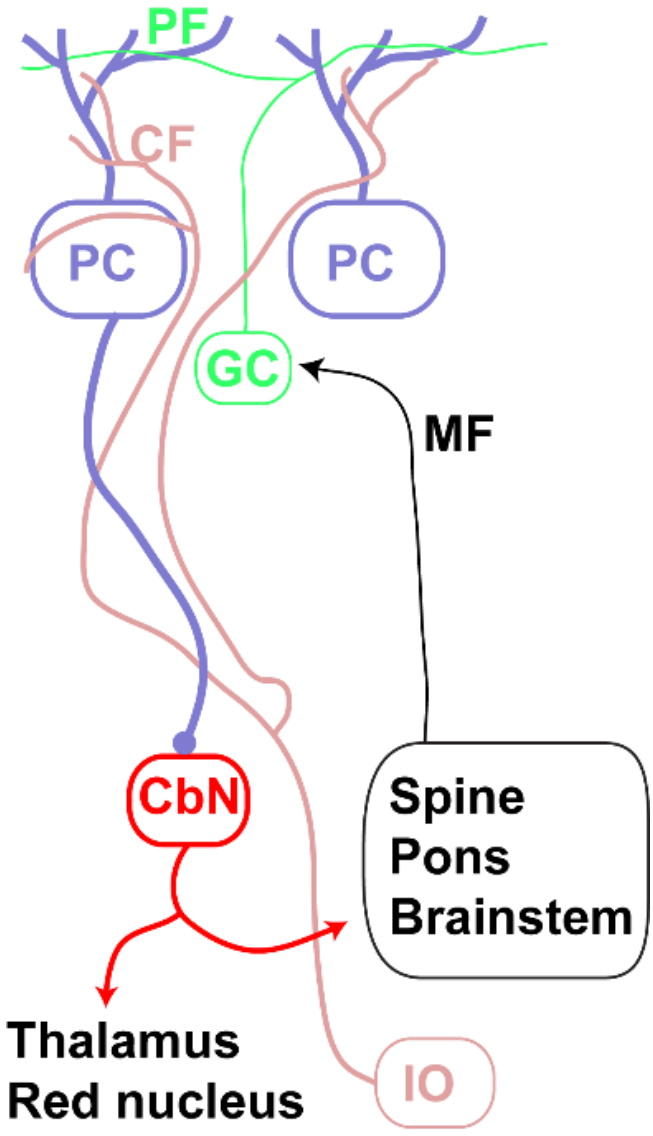

Figure 9. Canonical cerebellar microcircuit. Ascending sensory information enters the cerebellar cortex via mossy fibers (MFs) that excite granule cells (GCs). The axons of GCs ascend to the molecular layer where they run parallel to each other—hence the name parallel fibers (PFs)—to synapse on the dendritic trees of Purkinje cells (PCs), the cell type that is the sole output of cerebellar cortex. The other input to PCs are the climbing fibers (CFs) from the inferior olive (IO). PCs project to the cerebellar nuclei (CbN) where they inhibit the projection neurons that convey cerebellar output to the rest of the brain.

cerebellum, particularly the more lateral regions, may be involved with cognitive aspects of speech.

Since very early studies of brain function in the 1800s using pigeons, it has been known that the cerebellum is not required for movement but that damage to the cerebellum results in movements that are not coordinated (Flourens 1842). Similar results have been obtained using more modern methods for a variety of reflexes and learned motor skills, including many different types of eye movements, eyeblink as studied with classical conditioning, grasping and gripping, and speech as I will discuss in the section on motor control of speech below. In all cases, cerebellar damage does not prevent movement but it does result in uncoordinated movement and it can lead to a loss of the ability to modify movements based on context.

The anatomy of the cerebellum also supports the idea that it regulates fine control of movements (Apps and Garwicz 2005). All of cerebellar cortex consists of a crystal-like lattice built from just a handful of cell types (depicted in Figure 9), in spite of the fact that different regions of cerebellar cortex receive very different sensory inputs. These findings suggest that the cerebellum performs the same computation for all of the different behaviors it is involved in. The canonical cerebellar microcircuit begins with ascending sensory input that enters the cerebellar cortex via mossy fibers (MFs) from the spinal cord and hindbrain and midbrain regions, in particular the pontine nuclei that process input from many regions of cerebral cortex before passing it on to the cerebellar cortex (Brodal and Bjaalie 1992). MFs excite granule cells (GCs)—the most numerous cell type in the brain—who in turn send their axons up into the molecular layer where the axons form long parallel fibers (PFs). Between the granular layer and the molecular layers sits the Purkinje cell (PC) layer. The highly complex dendritic trees of PCs fan out orthogonal to PFs so that a single PF can synapse on the dendritic trees of multiple PCs. These synapses provide the pathway for ascending sensory information to reach PCs, being transmitted through GCs from MFs. The GC inputs elicit simple spikes (SSs) from PCs. In addition to the many synapses that PCs form with PFs, they receive input from climbing fibers (CFs), axons of neurons in the inferior olive (IO). Each CF axon terminal contacts a single PC at the proximal dendrite on the soma, and when it spikes it triggers a massive multi-phasic action potential known as a complex spike (CS). Most theories of cerebellar computation hinge on how CSs might convey a signal from the IO that induces plasticity to change the SS behavior of PCs. The neural activity of PCs represents the sole output of the cerebellar cortex. PCs provide the main source of input to neurons in the cerebellar nuclei (CbN) and the vestibular nuclei, both of which transmit the output of the cerebellum to the rest of the brain.

Notably, PC output is inhibitory. It is thought that pauses in PC activity disinhibit CbN neurons, allowing them to fire and drive downstream activity when appropriate.

I.2.A.5.a.1 Cerebellothalamic system: anatomy

The projection from the cerebellum to the thalamus is of particular interest because through this pathway cerebellar output can reach motor cortex and the basal ganglia. It has long been known that in mammals a massive projection arises from the cerebellar nuclei and crosses the midline to target the contralateral thalamus (Vogt 1909, Walker 1938, Percheron, Francois et al. 1996) . The projection arises mainly from the lateral cerebellar nuclei, although there is a strong contribution from the intermediate nuclei and some from the medial nuclei as well (Haroian, Massopust et al. 1978, Tracey, Asanuma et al. 1980, Sugimoto, Mizuno et al. 1981, Asanuma, Thach et al. 1983). (Here I am using general terms to describe the anatomy although specific terms are applied to different species, e.g. in primates the lateral cerebellar nucleus is called the dentate.) Most studies identify two principal targets of the cerebellar nuclei (CbN) in dorsal thalamus: a region in motor thalamus (VL_{Cb}) and a region in the intralaminar nuclei, the central lateral nucleus (CL) (see Figure 8). I will describe differences between these two regions that pertain to possible hypotheses about cerebellar-recipient thalamus in songbirds. It is of particular importance for the studies in this dissertation to consider differences between VL_{Cb} and CL because both have been proposed as pathways through which the cerebellum can communicate with the basal ganglia.

Results of tracer studies make clear that the primary target of CbN across mammalian species is a ventral and lateral region in thalamus (Haroian, Massopust et al. 1978, Sugimoto, Mizuno et al.

1981, Asanuma, Thach et al. 1983, Aumann, Rawson et al. 1994). Recall that this region is part of the ventral tier nuclei, considered motor thalamus precisely because their major afferents are from the cerebellum and the basal ganglia. Terminology can vary between species to refer to different parts of motor thalamus, so to refer to the cerebellar-recipient part of motor thalamus in mammals I use the abbreviation VL_{Cb}. Retrograde tracer studies of the projection from CbN to VL_{Cb} do not mention other afferents besides the brainstem projections (Newman and Ginsberg 1993) referenced above in the section on the intralaminar nuclei and a projection form the spinothalamic tract (Tracey, Asanuma et al. 1980, Stepniewska, Sakai et al. 2003, Craig 2008). As just stated, the other major target of CbN in thalamus is CL, one of the intralaminar nuclei. Recall that the CM-Pf complex in the posterior intralaminar nuclei is the main source of thalamostriatal projections, but that CL projects to striatum as well. Most tracing studies show less dense terminations from CbN in CL compared to VL_{Cb}. However, there does not appear to be in most species a distinct projection to CL; instead there is a gradient of CbN projection density that decreases moving from VL_{Cb} to CL (Sugimoto, Mizuno et al. 1981, Asanuma, Thach et al. 1983, Aumann, Rawson et al. 1994, cf. Fig. 1) (for examples of label in VL and CL resulting from tracer injections in CbN, see Figure 10 reproduced from Asanuma et al. 1983 and Figure 11 reproduced from Sugimoto et al. 1981). In addition to input from CbN, CL receives strong input from brainstem cholinergic cell groups, as do the other intralaminar nuclei. But again as discussed above it is the case that VL also receives cholinergic input and the difference is more of intensity than a clear-cut difference between VL_{Cb} and CL. There are also reports that CL like VL_{Cb} receives input from the spinothalamic tract (Ma, Peschanski et al. 1987) so that is probably not a clear way to differentiate between the two. Hallanger et al. in their study of subcortical inputs to thalamus in rats (Hallanger,

Levey et al. 1987) identified the ventral tegmental nucleus in the brainstem as a group of neurons that only targets CL. Their results also show strong label of superior colliculus after an injection in CL but not in VL. Other groups have shown that the superior colliculus seems to project specifically to CL as well (Graham 1977, Graham and Berman 1981). Again as stated above, CL and the intralaminar nuclei in general are thought to be driven by superior colliculus inputs, and the deep layers in particular are considered the source of multisensory responses that can be recorded from the intralaminar nuclei (Smith, Galvan et al. 2014).

Regarding the structure of cerebellar synapses in thalamus and the neurotransmitter they use, most analysis has focused on VL_{Cb}. Unlike pallidal-recipient thalamus, the cerebellar input to thalamus is glutamatergic, i.e. excitatory, and most structural studies agree that cerebellar input should be the primary driver of ventral thalamic neurons (Rinvik and Grofova 1974, Harding and Powell 1977, Aumann, Rawson et al. 1994, Kuramoto, Fujiyama et al. 2011). As with pallidal-recipient thalamus, there is a cortical input to cerebellar-recipient motor thalamus, but the morphology of these terminals suggests they are modulators and not drivers of this part of thalamus (Harding and Powell 1977, Kuramoto, Fujiyama et al. 2011). EM results also show the smaller corticothalamic terminals form on distal dendrites while cerebellar inputs form on proximal dendrites (Ilinsky 1990), consistent with the idea that cortical inputs would modulate the drive from cerebellum.

I now discuss in some detail the evidence supporting disynaptic pathways from CbN to striatum through both VL_{Cb} and CL in mammals, because if there is more evidence for one pathway or another, then this should inform hypotheses about a similar pathway in songbirds. The first evidence for a disynaptic pathway from CbN through thalamus to striatum was shown in rats,

where the thalamic nucleus was identified as CL (Ichinohe, Mori et al. 2000). In this study, axon terminals from CbN are shown to form terminals on dendrites of thalamic neurons. This is shown with light microscopy and EM images from paired tracer injections of CtB in dorsolateral striatum and biotinylated dextrans or WGA-HRP in the cerebellar nuclei. Schematic drawings of their results show that most anterograde label from CbN is in VL and CL, and that most of the retrogradely labeled neurons are in CL and in Pf. Although that study did not show whether CL neurons project to striatum (e.g. with anterograde label), their results are consistent with other reports from single-axon tracing studies that show that CL neurons send collaterals throughout the striatum (Deschenes, Bourassa et al. 1996) and that they likely target MSNs (Ichinohe, Iwatsuki et al. 2001). The next major study to report that the cerebellum communicates with the basal ganglia was based on experiments with transsynaptic tracers in primates (Hoshi, Tremblay et al. 2005). The main experiment showed that injections in the striatum strongly labeled the cerebellar nuclei after allowing for "second order" infection, i.e. for the tracer to jump one synapse from thalamus to the cerebellar nuclei. Follow-up experiments reported that both the intralaminar and ventral motor thalamic regions showed "second order" labeling of neurons after transynaptic tracer injections into GPe, suggesting that both regions of thalamus communicate with the basal ganglia. Of course this latter result does not demonstrate through which thalamic nucleus the cerebellar nuclei reach the striatum. A recent study using highly dilute virus to label single neurons in rat thalamus found that neurons in VL_{Cb} project only to cortex, and that only the basal ganglia-recipient part of VL contains neurons that send out thalamostriatal collaterals (Kuramoto, Furuta et al. 2009). The authors identify cerebellar and basal ganglia recipient regions of motor thalamus by labeling an "inhibitory zone" with antibodies against one isoform of the GABA synthesis

enzyme, GAD67, and labeling an “excitatory zone” with antibodies against vesicular glutamate transporter VGLUT2. Previous work (Schwarz and Schmitz 1997) as well as their own follow-up studies (Kuramoto, Fujiyama et al. 2011) show that the “inhibitory zone” as labeled with GABA or GAD67 corresponds to the region of VL that receives basal ganglia input, while the “excitatory zone” as labeled with VGLUT2 corresponds to the region of VL that receives cerebellar input. Other groups have taken this same approach to delineate the pallidal-recipient and cerebellar-recipient regions of motor thalamus (Nakamura, Sharott et al. 2014). The Kuramoto et al. study’s finding that neurons in the excitatory cerebellar-recipient part of motor thalamus project exclusively to cortex suggests that CL is then the main thalamic nucleus through which the cerebellum communicates with the basal ganglia. The only other single-axon tracing study of VL I find reported that, in rats, one of eleven neurons had a branch into striatum (which they were unable to trace to its terminal) but did not comment further (Aumann, Ivanusic et al. 1998).

It is also relevant to the studies in this dissertation to ask how the projection of CbN to thalamus relates to the projection of CbN to the red nucleus. If cerebellar output in songbirds seems to reach the song system through thalamus, but is also sent to the red nucleus as it is in mammals, this raises the question of whether the red nucleus is somehow involved with birdsong. In the hierarchical framework of motor control, it is neurons in motor cortex that integrate input from the cerebellum via thalamus and then modulate activity of the red nucleus and other lower brain structures as well as motor neurons. However if single neurons in CbN project to both motor thalamus and red nucleus, it is possible the red nucleus receives copies of what CbN communicates to thalamus. One study that reconstructed single axons from rat CbN neurons found that lateral CbN neurons

projecting to thalamus also sent collaterals to red nucleus (Shinoda, Futami et al. 1988). These results alone do not show whether all cerebellothalamic neurons send collaterals to the red nucleus, or whether there are distinct populations in CbN that project to one or the other region. One approach to answer this question would be to inject retrograde tracers in both thalamus and the red nucleus and then quantify double-labeled neurons in CbN but I do not find any such study. This could be technically challenging given the proximity of the two regions.

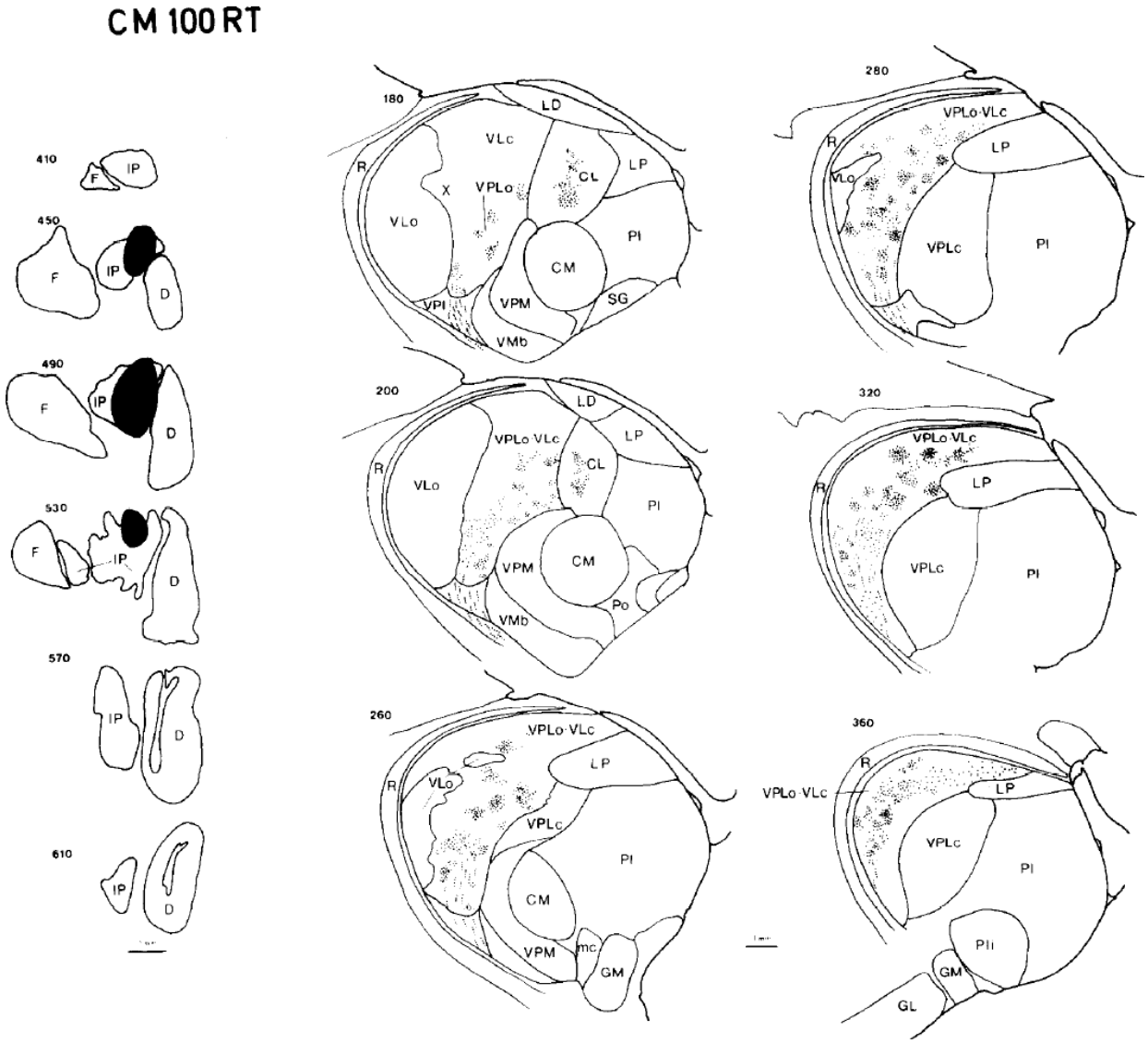

Figure 10. Cerebellar-recipient thalamus in a cynomolgus monkey Projection drawings of parasagittal sections through the thalamus of a cynomolgus monkey following a limited injection of tritiated amino acids into the interposed nucleus demonstrating the continuity of labeling patterns across the VPLo and VLc nuclei. Additional terminal labeling occurs in CL.

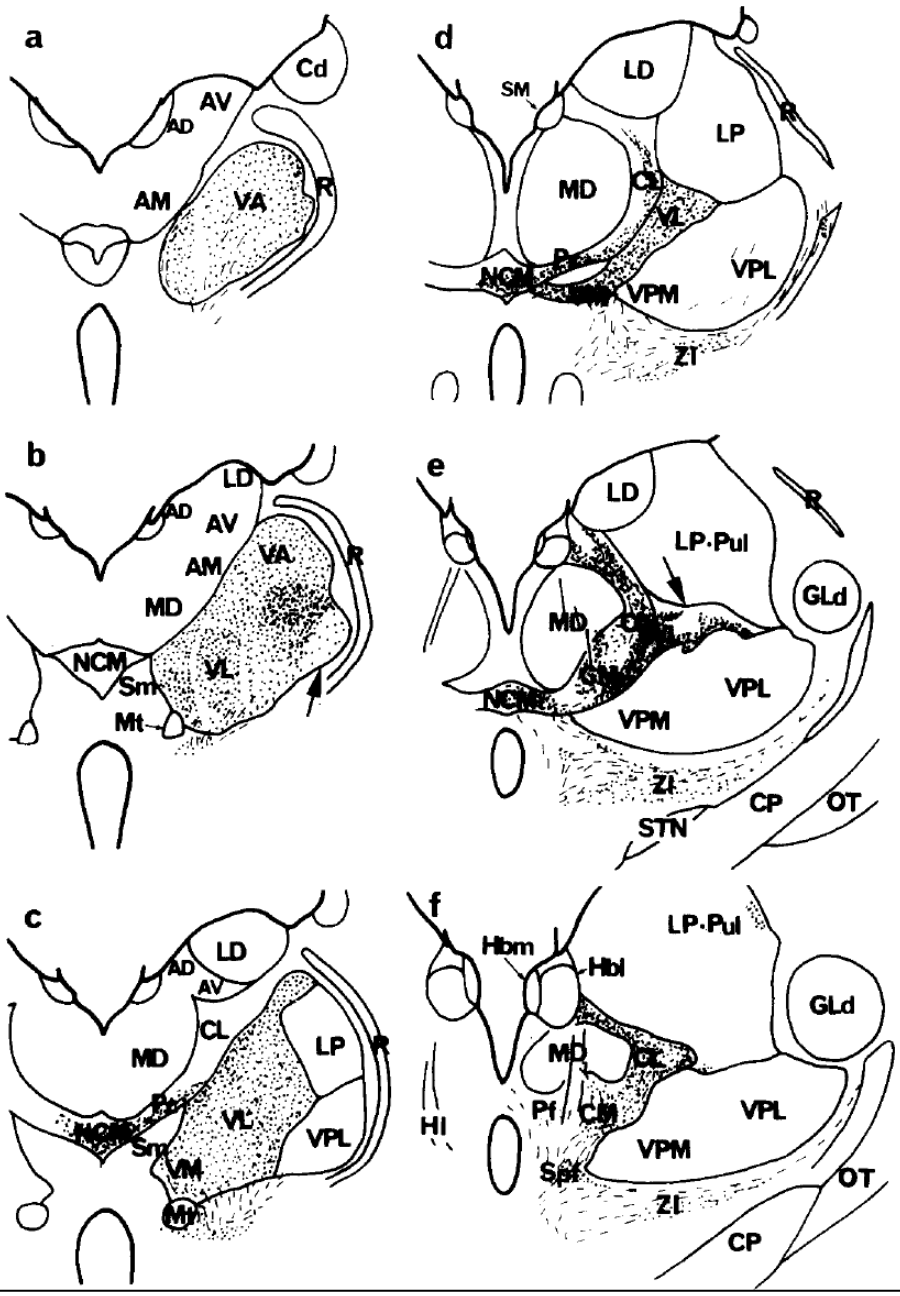

Figure 11. Cerebellar-recipient thalamus in a cat Projection drawings of frontal sections of thalamus, showing distribution of label after injecting isotope contralaterally in all cerebellar nuclei in Cat 4. Probable terminal silver grains and labeled fibers of passage are represented respectively with dots and broken lines in this and all subsequent figures. The drawings are arranged from rostral (a) to caudal (f). Reproduce from (Sugimoto, Mizuno et al. 1981) with permission.

I.2.A.5.a.2 Cerebellothalamic system: physiology and proposed function

If there is a region of thalamus in songbirds that receives input from the cerebellum and projects to song system nuclei in the forebrain, an obvious question would be what function is provided by cerebellar input. One way to answer this question would be to investigate how cerebellar input modulates activity in dorsal thalamus during song. To provide background on this question, I review studies of how cerebellar inputs modulate activity in motor thalamus during movement in mammals. However--unlike studies of basal ganglia output to motor thalamus that ask how firing from the upstream area might regulate spiking —most physiological studies of cerebellar thalamus have investigated what aspects of firing patterns best correlate with movement. Most of these studies have focused on motor thalamus, i.e. VL_{Cb}, not the cerebellar-recipient intralaminar nucleus CL. Recall also that, while input from the basal ganglia to thalamus is GABAergic and inhibitory, input from cerebellum to thalamus is glutamatergic. In fact, stimulation in the cerebellar nuclei results in large excitatory post-synaptic potentials in the thalamic neurons they target (Uno, Yoshida et al. 1970, Shinoda, Futami et al. 1985), so unlike the basal ganglia, the cerebellum clearly drives firing in motor thalamus. Almost all studies were performed with primates performing various arm movement tasks. Functions that have been proposed for the cerebellothalamic pathway include: movement initiation, control of movement parameters, timing, and reflex gain.

Arguments for a role of the cerebellum in movement initiation (Thach 2014) are based on the following evidence: (1) cerebellar damage impairs movement, (2) cerebellar stimulation can cause movement, and (3) cerebellar activity precedes motor cortical activity. As with the basal ganglia,

effects of damage to the cerebellum may not reflect function but instead may arise as a consequence of other brain areas compensating for loss of function. Similarly the effects of stimulation may result from effects on downstream areas when they receive output from the cerebellum that is not physiologically realistic. As for whether cerebellar activity precedes that of cortex, other authors report that cerebellar thalamus in particular fires after cortex, as referenced below. A function for the cerebellum in initiating movement is not mutually exclusive with it playing a role in on-line control of movement kinematics, and even those who argue for its role in movement initiation recognize that there is significant evidence in support of the cerebellum coordinating movements through some sort of model that can be modified by experience (Thach 2014).

Some authors hold that the difference between how the cerebellum and the basal ganglia contribute to movement lies in whether the movement is externally or internally guided (Sommer 2003). In spite of a difference in terms, this view is somewhat similar to the idea that the cerebellum builds a predictive model while the basal ganglia perform a reward-like computation. There are several studies of whether cerebellar thalamus contributes to externally-guided movements. Miall and co-workers studied both cerebellar-recipient and pallidal-recipient motor thalamus with single unit recordings (Van Donkelaar, Stein et al. 1999) and pharmacological inactivation (Van Donkelaar, Stein et al. 2000). In the experiments, monkeys made reaching movements, either visually guided towards a target or internally generated—i.e., initiating each trial after waiting a minimum time instead of waiting for a target to appear. Neural recordings showed that thalamic neurons in the cerebellar-recipient areas were more likely to significantly change their activity during the task

with a visual target than during the internally-generated movements. Inactivation of cerebellar-recipient thalamus increased reaction times and impaired kinematics specifically on the visually-guided trials. Miall et al. concluded that cerebellar-recipient thalamus is more involved with what they termed "visually triggered" movements. As the authors discuss, the results agree with those of Mushiake and Strick who made single unit recordings from GP (Mushiake and Strick 1995) and CbN (Mushiake and Strick 1993) during a sequential pointing task. Mushiake and Strick found a region in the dentate where activity correlated with the task when it was visually guided, while GP activity correlated best with the task when the monkey had to recall it from memory.

One view is that there is little evidence for any of the functions listed above (Horne and Butler 1995). A role in movement initiation can be ruled out based on recordings from wrist-related neurons in cerebellar thalamus and cortex showing that cortical activity preceded activity in cerebellar thalamus (Butler, Horne et al. 1992). The same recordings do provide some support for the possibility that the cerebellum contributes to timing: the peak of cerebellar thalamic activity occurred at a time that could drive motor cortical activity. However the behavioral task was not designed to explicitly test how timing of cortical activity depended on thalamic activity. With regard to the idea of gain control, another study tested whether activity in the cerebellar thalamus could modulate a reflex-like "loop" through cortex (Butler, Horne et al. 1992). It has been hypothesized that motor cortex and cerebellum might be involved in such a loop that would dampen oscillations that arise due to feedback, by analogy to how the cerebellum modulates the gain of the vestibulo-ocular reflex. However, spiking activity in cerebellar thalamus did not correlate with EMG oscillations (Butler, Horne et al. 1992). Hence that study did not find evidence

for a reflex gain function for the cerebellothalamic pathway. Yet another proposed function of the cerebellar output is to encode movement parameters such as direction, force, or kinematics. Firing patterns of neurons in cerebellar thalamus appear to have a "preferred" direction (Butler, Horne et al. 1992), similar to motor cortical units, but the activity of these neurons does not correlate with force (Butler, Horne et al. 1992), and it is not clear whether their activity encodes kinematic parameters such as velocity (Horne and Butler 1995).

Trial-by-trial analysis of firing rate and movement parameters found low correlations during voluntary movement but an increase in correlation when a perturbation was applied (Butler, Finkelstein et al. 1996). They conclude that the evidence is most consistent with the function of cerebellar thalamus being to relay error signals that the cerebellum emits when it detects a mismatch between efference copy and actual motor output (Horne and Butler 1995). Of course this sort of error detection is often taken as a prerequisite for the brain to build and maintain an internal model. A similar analysis of single units in cerebellar thalamus during a sensorimotor adaptation paradigm provided additional support for the idea that this pathway relays error signals (Butler, Bourke et al. 2000). Recordings from a new paradigm designed so that force and velocity would not covary again found little evidence for force or kinematic information on a trial-by-trial basis (Ivanusic, Bourke et al. 2005). The authors devised a post-hoc analysis of ensembles and found that at a population level the neurons best represented duration. Based on this result they concluded that cerebellar thalamus probably conveys timing information to motor cortex.

It is difficult at present to see a clear picture emerging across studies from different groups that I have just reviewed. Most studies do not report clear large-magnitude correlations between any

parameters of movement such as force or kinematics (e.g. velocity). As others have noted, this lack of strong evidence for the cerebellothalamic pathway encoding movement parameters given, for example, many results showing Purkinje cells in cerebellar cortex do encode movement kinematics (Ebner, Hewitt et al. 2011). This lack of clear findings may arise in part from methodologies. For instance, although the Butler group is careful to look for correlations on a per trial basis (instead of averaging across all trials), they often make use of derived metrics like mean firing rate. The studies cited are also careful to anatomically and physiologically identify recording targets—e.g. they target the known wrist region of cerebellar thalamus and look for somatosensory responses from neurons before recording during tasks that involve wrist flexion—but it is possible they conflated recordings from the multiple regions in thalamus representing any given body part. Recall that Strick and co-workers have identified multiple representations of the body in the cerebellar nuclei corresponding to the different regions of motor cortex: i.e. there is one region in dentate labeled by trans-synaptic tracer after injections in the arm area of primary motor cortex, and another, largely non-overlapping region in dentate that is labeled after injections in the arm area of supplementary motor cortex (Middleton and Strick 2000). (Presumably these tracing results can only arise if there are similar multiple representations in thalamus.) Yet another caveat is that differences in behavioral paradigms can affect whether correlations with kinematics are found in single-unit recordings from the cerebellum (Ebner, Hewitt et al. 2011) and hence could explain why they were not seen in recordings from cerebellar-recipient thalamus as well. Future studies should employ tasks that previous work suggests is more likely to involve the cerebellum, such as multi-joint movements (Ebner, Hewitt et al. 2011). These studies (in primates and in songbirds) may also benefit from analyses that compare, at each time point in a trial, the instantaneous firing

rate with the value of the movement parameter(s) being investigated. Such analyses may help avoid the difficult-to-interpret correlations reported in the studies reviewed that were based on mean firing rates. Lastly, more studies from multiple groups should be explicitly designed to differentiate between proposed functions such as movement initiation, timing, and encoding force or kinematics.

I.2.A.5.a.3 Other relevant cerebellar pathways

The cerebellothalamic pathway is most relevant to the anatomical questions addressed in this dissertation, but there are other pathways through which the cerebellum is likely to be involved with vocalization. To vocalize, the brain must co-ordinate respiration with movement of the vocal organ and of articulators such as the jaw and tongue. Evidence that the cerebellum contributes to respiration and orofacial control comes from anatomical as well as physiological and behavioral studies.

Evidence for a cerebellar role in respiration comes mainly from studies of the medial cerebellar nuclei, i.e. the fastigial nucleus (FN) (Xu and Frazier 2002). Tracing studies show that the fastigial nucleus projects contralaterally to several nuclei in the pons and medulla involved with respiration (Xu and Frazier 2002). Two such regions whose regulation by FN has been studied directly are the medullary gigantocellular nucleus (Gi) (Xu, Zhou et al. 2001) and the ventromedial medullary reticular formation (Lu, Cao et al. 2013). Xu et al. (2001) injected tracer in Gi and retrogradely labeled neurons in FN, and showed that bilateral chemical lesions of Gi block changes to respiration that can be induced by FN stimulation in rats. Similarly in mice, Lu et al. (2013) showed

by making BDA injections in FN that it projects mainly to the contralateral Gi, although they also noted label in neighboring regions of the medullary reticular formation. As for electrophysiological evidence that FN is involved with respiration, Lu et al. also report a correlation of respiratory rhythm with spike timing of single units in FN, and Gruart and Delgado-Garcia (1992) observed single units in FN of cats whose firing rate varied in sync with the phase of the respiratory cycle. Other studies have provided similar evidence (Lutherer, Williams et al. 1989, Xu and Frazier 1997). In terms of function of the pathways from FN to brainstem, the bulk of the evidence points towards a role for FN not during normal breathing but during "stressed" breathing. Most studies report no effect on eupneic (i.e. normal, resting) breathing after lesion of FN or ablation of relevant cerebellar regions. However stimulation of FN can affect respiratory rhythm, and respiratory challenges can change firing of single units in FN so that it correlates with the breath cycle. A pharmacological study also showed that increased carbon dioxide in FN but not other regions of the cerebellar nuclei increased respiratory output (Xu, Zhang et al. 2001). This result also supports the view that the FN is chemosensitive, and so changes in blood carbon dioxide levels (i.e bicarbonate) would recruit the cerebellum to modulate breathing. It is likely that any prolonged vocalization like birdsong or speech would necessitate such a change in the concentraiton of circulating carbon dioxide.

Anatomical studies provide evidence that sensory input from the jaw (Jacquin, Semba et al. 1982, Huerta, Frankfurter et al. 1983). tongue, and larynx reaches cerebellar cortex. However there is little evidence that the cerebellar nuclei project directly to brainstem motor nuclei involved with control of the jaw, tongue, and larynx. It has also been reported that specific regions of cerebellar

cortex and FN receive input from motor neurons in the relevant brainstem nuclei, raising the possibility that the cerebellum receives efference copies of motor commands during vocalization. Evidence for cerebellar involvement in jaw movement comes from microstimulation studies of the rat cerebellar nuclei. It should be pointed out that, since all regions of the cerebellar nuclei project to thalamus, it is possible that a neuron for example in FN may project to both brainstem motor nuclei and to motor thalamic regions that project to cortex. In this way the orofacial regions of motor cortex could receive copies of the output the cerebellum sends to brainstem motor neurons. I do not find previous studies that consider this possibility e.g. with paired retrograde tracer injections in thalamus and brainstem.

I.2.A.5.a.4 Cerebellar function in speech

Lastly I outline proposed functions of the cerebellum in speech. Because so little is known about how the cerebellum is involved with speech, I introduce this in a section separate from questions of anatomy. Almost all proposed functions are based on speech deficits that result from cerebellar damage, for example due to stroke or neurodegenerative disease. Typically any speech disorder associated with cerebellar insult is classified as ataxic dysarthria. Perhaps because this diagnosis is typically applied after a patient is known to have suffered cerebellar damage, it does not seem to be defined by a clear set of symptoms. Multiple subtypes are recognized. Recent reviews state that the most common symptoms relate to respiration and phonation (Marien, Ackermann et al. 2014). Many authors describe increased duration of speech segments and “scanning” speech (Darley, Aronson et al. 1975, Kent, Kent et al. 2000), but as others point out both of these symptoms can arise in other speech disorders, and so they may just generally occur when the motor

system is impaired, or they may arise as the brain tries to compensate for damage (Marien, Ackermann et al. 2014). Speaking task studies do show that diadochokinesis (rapid repetition of a syllable) is specifically impaired in ataxic dysarthria but not speech apraxia (Kent, Kent et al. 1997, Ziegler 2002). Neurologists conceive of ataxic dysarthria as a disorder of production, as distinguished from motor planning/programming. This has not prevented authors from assigning the cerebellum a function in speech similar the functions it is proposed to have for other movements. The impaired ability to change rate of syllable repetition has been taken as evidence that the cerebellum participates in feedforward planning of speech (Spencer and Slocomb 2007). There are some recent lesion mapping studies that more carefully relate areas of cerebellar damage to speech disorder symptoms. The results localize articulation symptoms to the rostral paravermal area of the anterior lobe (Urban, Marx et al. 2003). Another larger study supported this finding in that they found dysarthria specifically associated with lesions of paravermal and hemispheral lobules V and VI (Schoch, Dimitrova et al. 2006). Not to belabor the point, but ataxic dysarthria symptoms are of course consistent with effects that cerebellar damage has on other movements, and again the same caveats apply when extrapolating from these symptoms to possible functions. I.e., just because speech segments are elongated after cerebellar damage, it does not mean the cerebellum is required for speech initiation, because the same symptom might be explained by diaschisis in cortex simply because it receives less “drive” after cerebellar damage. These problems with interpretation illustrate the need for an animal model to more precisely investigate possible roles of the cerebellum in vocalization.

I.2.A.5.b Possible interactions between the basal ganglia and cerebellum

It has been convenient and scientifically productive to focus on the basal ganglia as the site of reinforcement learning and the cerebellum as the site of sensorimotor adaptation (Shmuelof and Krakauer 2011). This made sense especially in the view of motor systems where each subcortical structure communicated via segregated pathways with motor cortex through thalamus. Nevertheless, as discussed in section I.2.A.5.a.1, more and more evidence suggests these two subcortical structures can communicate directly (Ichinohe, Mori et al. 2000, Hoshi, Tremblay et al. 2005, Jinnah and Hess 2006, Neychev, Fan et al. 2008, Chen, Fremont et al. 2014). This communication between the two structures raises questions about the extent to which they interact and the extent to which they independently carry out computations. The concept applied to the basal ganglia of multiple parallel and re-entrant loops has been extended to include the cerebellum. This is based in part on transsynpatic tracer studies referenced earlier that demonstrate multiple representations of muscles in cortex—e.g., there is an arm area in motor cortex, premotor cortex, and supplementary motor cortex—and that each of these regions communicates with distinct regions in the basal ganglia and cerebellum (Middleton and Strick 2000). To my knowledge, only one physiological study of the cerebellum-to-basal ganglia pathway has been carried out. The authors found mixed effects on the firing rate of MSNs when stimulating the cerebellar nuclei electrically or optogenetically in freely-behaving rats (Chen, Fremont et al. 2014): some MSNs were excited, some were inhibited, and some had multi-phasic responses. Excitatory and inhibitory responses occurred at a latency consistent with a disynaptic pathway. Pharmacological inactivation demonstrated that these effects were in fact mediated by CL, the main cerebellar-recipient region

of the intralaminar nuclei. The authors also provided evidence *in vivo* that paired high-frequency stimulation of the cortical and cerebellar inputs to striatum yields long term potentiation of the corticostriatal synapses. High-frequency stimulation of corticostriatal input alone resulted in long-term depression at the corticostriatal synapse; hence, the authors suggest cerebellar input could guide the sign of plasticity at corticostriatal synapses. With respect to a function for the cerebellar-to-basal ganglia pathway, one study proposes that error signals from the cerebellum may gate temporally-delayed reward predictions in the basal ganglia (McDougle, Boggess et al. 2016). They found that such a model best explained results from a study designed so that movement errors were linked with outcomes on a gambling task: when the task required reaching movement where the experimenters could artificially impose errors, subjects were more likely to make risky bets, as if they were biased to assign the outcomes to the perceived movement errors (McDougle, Boggess et al. 2016). This function may make the most sense for the pathway to striatum through CL, given the findings from electrophysiological studies of the cerebellothalamic pathway that perturbations—presumably inducing error—had the greatest effect on activity in that pathway.

I.2.A.5.c Motor control of speech

Lastly before I turn to songbirds I outline what is known about motor control of speech, with a focus on other pathways through which the cerebellum in particular might contribute to vocalization. I neglect classical models of speech that emphasized cortical interactions between Broca's area and Wernicke's area via the arcuate fasciculus (Geschwind 1972), but instead describe the descending corticospinal pathways from motor cortex. That is because generally speaking in mammalian motor systems the cerebellum is known to be interconnected with the

descending pathways. Below (in section I.2.D.1) I describe similar descending projections (Wild 1992, Wild 1997, Wild and Williams 1999, Wild and Williams 2000), thought to be like the corticospinal tracts in mammals, that my results show are also interconnected with the cerebellum in a songbird (Appendix V.2.C, Figure 27, Figure 28, Figure 29, Figure 30). A representation of the vocal motor system in humans is shown in Figure 12. Ultimately to produce speech the brain must control the muscles of the larynx, the vocal organ, in coordination with effectors like the tongue and jaw as well as with the respiratory system. The muscles controlling the larynx, effectors, and respiratory system are innervated by motor neurons in the brainstem and dorsal spinal cord (Jürgens 2002, Jürgens 2009). Laryngeal motor neurons reside in the nucleus ambiguus, while motor neurons controlling the jaw and tongue are found in the hypogolossal, facial, and trigeminal nerves, and respiratory muscle motor neurons are located in the spinal cord. Neurons in these cranial nerves and spinal cord ganglia do not directly project to each other to co-ordinate movement. Instead, as shown by tracing studies in spider monkeys (Thoms and Jürgens

1987), they all receive input from a network of interneurons in the brainstem. The network consists of neurons found in specific subregions of the parabrachial area, the reticular formation, and nucleus retroambigualis. In turn this network receives projections from laryngeal motor cortex (Simonyan and Jürgens 2003). Laryngeal motor cortex forms reciprocal connections with ventrolateral thalamus (Simonyan and Jürgens 2003, Simonyan and Jürgens 2005), and in this way can integrate output from specific parts of the basal ganglia and cerebellum (Jürgens 2009). There are other pathways (not shown in figure) that play important roles in speech. In addition to projecting to the reticular formation, laryngeal motor neurons project to a midbrain region, the periaqueductal gray (PAG), that also projects upon nucleus ambiguus as well as nucleus retroambigualis, a brainstem region that plays an important role in respiration. In all

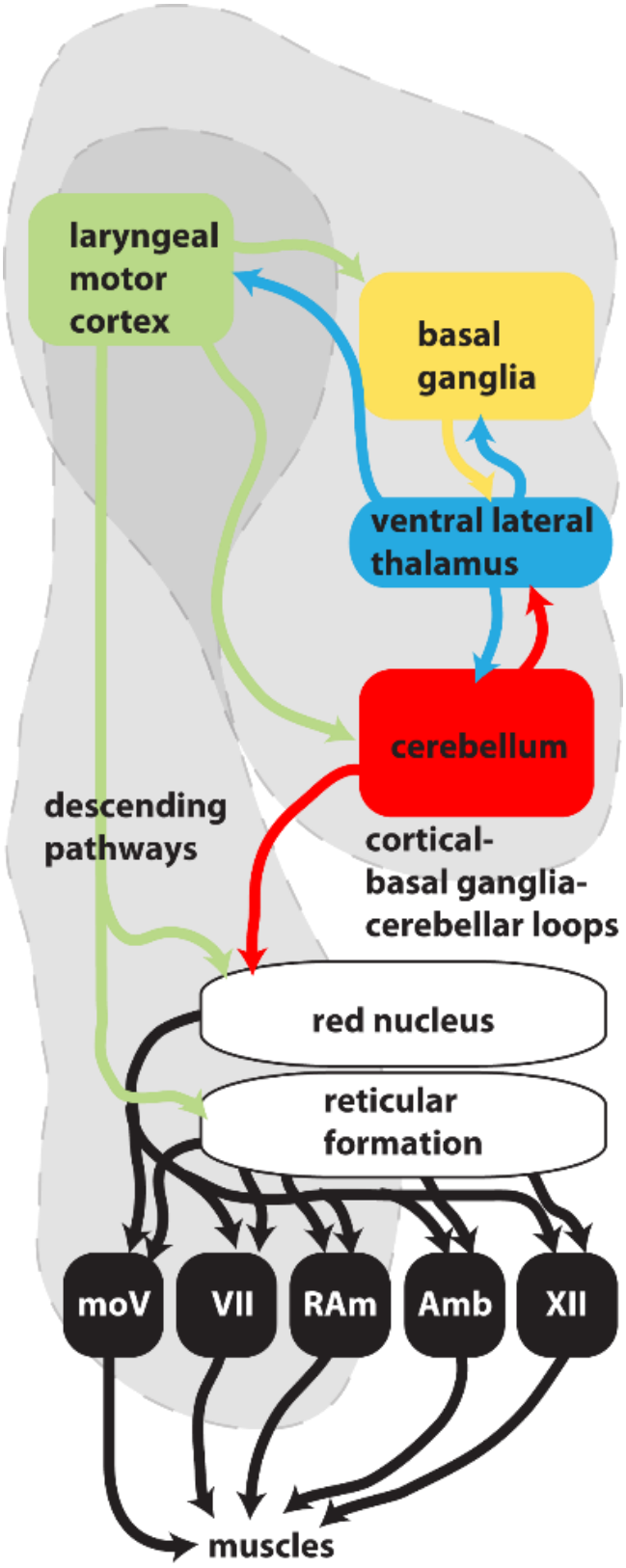

Figure 12. Speech motor control. Description in text.

mammals tested, stimulation of PAG can elicit vocalizations, as can stimulation of anterior cingulate cortex (ACC) in primates, a cortical region thought to regulate “emotional utterances”, i.e. non-speech vocalizations like cries. Most workers agree that the projections from ACC and from laryngeal motor cortex form two separate pathways for vocal control (Jürgens 2009, Belyk and Brown 2017). Recent work with humans has identified two separate laryngeal motor cortex regions, one of which appears to have evolved specifically in great apes and expanded in humans to afford the control required for language (Belyk and Brown 2017). ACC also projects to PAG. In addition to its connections with nucleus ambiguus, PAG sends efferents to nucleus retroambigualis, another brain stem group of MNs that in this case controls muscles involved in respiration such as the diaphragm.

Are there pathways through which the cerebellum might interact with any of the brainstem and midbrain regions also targeted by laryngeal motor cortex? In their study of the cranial nerves, Thoms and Jürgens (1987) found that nucleus ambiguus did receive projections from the fastigial nucleus (medial cerebellar nucleus), but none of the other nuclei did. Other studies have reported retrograde label of motor neurons e.g. in the trigeminal nucleus from tracer injections in cerebellar cortex, suggesting these motor nuclei might send an efference copy-like signal to cerebellum (Kotchabhakdi and Walberg 1977, Saigal, Karamanlidis et al. 1980, Bukowska and Grottel 1997). There does not seem to be evidence that the region in the reticular formation that projects to all the phonatory nuclei interacts monosynaptically with the cerebellum. In other words, there is little support for the idea that the cerebellum directly interacts with the phonatory nuclei or the reticular formation region that co-ordinates their activity. However, Thoms and Jürgens (1987) did show

that all of the phonatory nuclei receive input from the red nucleus, which in turn gets cerebellar and cortical output. In monkeys the cortical projections target the parvocellular division of the red nucleus, as does the dentate of the cerebellar nuclei, while the interpositus cerebellar nuclei targets the larger magnocellular division of the red nucleus (Kennedy, Gibson et al. 1986). One group found that stimulation of a dorsolateral portion of the magnocellular division elicited mouth movements and recorded single-unit activity there that correlated with orofacial movement (Kennedy, Gibson et al. 1986), consistent with the anatomical results of Thom and Jürgens. Others have also confirmed with tracer studies of nucleus retroambigualis that it receives projections from the red nucleus (Vanderhorst, Terasawa et al. 2000). Note that this nucleus exerts massive control over respiration and project directly to ambiguus, the nucleus that controls the laryngeal muscles, in primates (VanderHorst, Terasawa et al. 2001). Similarly nucleus retroambigualis in songbirds is reciprocally connected with the hypoglossal nucleus in songbirds that innervates the muscles of the syrinx (Wild, Williams et al. 2000). In addition to the pathway through the red nucleus that might allow the cerebellum to modulate vocalization, there is some evidence for direct interactions.

I.2.B Open question about the song system: does the song system contain a thalamostriatal projection?

Although the connectivity of the AFP has been studied in detail, important questions remain unanswered. One such question is whether Area X receives input from the thalamus (Gale and Perkel 2010). In mammals, the striatum receives massive input from the thalamus, but the function of this thalamostriatal system remains poorly understood. If similar projections were to exist in the

AFP, it might make possible comparative studies that would inform our understanding of basal ganglia function in the same way previous work has.

I.2.B.1.a Evidence for a thalamostriatal system in birds

As described above, in the classic hierarchical view of the motor system, both the basal ganglia and the cerebellum communicate with motor cortical regions via their output to the so-called motor thalamus, a set of ventrally-located thalamic nuclei. However both the basal ganglia and the cerebellum also project to the intralaminar and midline thalamic nuclei, the main source of the thalamostriatal projection through which the cerebellum can interact with the basal ganglia.

Generally speaking, thalamus is not as well studied in birds as it is in mammals. DLM, the thalamic nucleus in the song system, is found in dorsal thalamus (sometimes called the dorsal thalamic zone, I will use the abbreviation DT). In the songbird AFP, nucleus DLM is the thalamic component of the thalamocortical-basal ganglia loop. It is not at all clear whether DLM is like motor thalamus or the intralaminar and midline nuclei. I outline the anatomical and physiological evidence and then present what is known about the physiology of DLM as it relates to the anatomical questions addressed in this dissertation.

I first review what is known about dorsal thalamus in birds in general, and then I discuss results in songbirds as they relate to the song system. Many of these results may seem tangential to the question of whether there is a thalamostriatal projection in the song system. I emphasize that most studies of the thalamostriatal system in mammals find clear differences between striatal projections from the intralaminar nuclei, in particular the CM/Pf complex, and striatal projections from other

regions in thalamus. Therefore it is important to carefully weigh the evidence for or against an intralaminar thalamus-like region in birds. The results are presented roughly in chronological order to give a sense of how thinking about how this brain area has changed. In their landmark study of the pigeon basal ganglia, Karten and Dubbledam (1973) identified the targets of the avian globus pallidus and discussed their possible homologies. One such target was DIP in dorsal thalamus, but Karten and Dubbeldam observed that GP also projected to the lateral spiriform nucleus (SpL) and the ansa lenticuluaris (AL). It is now generally accepted that AL is the avian subthalamic nucleus (Jiao, Medina et al. 2000). SpL receives a massive projection from GP, is situated more ventrally in thalamus than DIP, and itself projects almost exclusively to the optic tectum, the avian equivalent of the superior colliculus (Reiner, Brecha et al. 1982). Based on these facts alone, the SpL might be considered more like the ventral motor thalamus. However DIP also receives output from GP, and especially at the time of Karten and Dubbeldam's study, there was little data to differentiate SpL from DIP. In their discussion, Karten and Dubbeldam state that DIP is also the target of the cerebellar nuclei, suggesting that this might make it the homolog of the ventral motor nuclei.

Building on this work, Kitt and Brauth (1982) studied the projections of DIP and neighboring regions of dorsal thalamus with tritiated amino acids. They report that they confirmed the finding that GP projects to DIP, and in addition showed that DIP projects to a region of nidopallium. Based on these results they conclude that the GP-->DIP-->Nidopallium pathway is similar to the GP-->VA/VL-->M1 pathway in mammals. Wild further studied the projections of dorsal thalamus in pigeons with wheat germ agglutinin-horseradish peroxidase. He found that distinct regions of

dorsal thalamus projected not only to distinct regions of cortex but also to distinct regions of the striatum (Wild 1987). In his discussion, Wild allowed that avian dorsal thalamus could be like the intralaminar/midline nuclei or like the ventral tier motor nuclei.

If DIP were itself like ventral motor thalamus in mammals, then it would receive output from both the basal ganglia and the cerebellar nuclei. Arends and Zeigler (Arends and Zeigler 1991) carried out the first and to date only detailed study of the projections of the cerebellar nuclei in a bird. Their studies using HRP in pigeons did show that a projection to dorsal thalamus arises largely from lateral CbN. However they conclude that DIP is not the principal target of CbN. By comparing areas in dorsal thalamus that receive output from CbL with regions that receive output from the vestibular nuclei or from dorsal column nuclei, they show that the principal target of CbL is a rostral part of the region labeled DLP in the Karten and Hodos pigeon atlas. (Others had reported that the same region was the principal target of vestibular nuclei in thalamus (Wild 1988), hence the need to compare results.) The fact that the more caudal region of DLP receives only tectal output (Gamlin and Cohen 1986) further supports the idea that a distinct cerebellar-recipient zone in dorsal thalamus can be recognized. Arends and Zeigler proposed naming this area DLI to differentiate it from DIP and caudal DLP, but the name has not been adopted.

Arends and Zeigler's study provided further proof that avian dorsal thalamus, like mammalian thalamus, receives output from GP and CbN, and that these zones are largely segregated. However this leaves unresolved the question of whether dorsal thalamus is more like the ventral tier motor nuclei or like the intralaminar nuclei. Medina et al. (1997) report that they identified a region outside dorsal thalamus, the ventral intermediate area (VIA), that they argue is like the ventral

motor nuclei. They show with tracer injections that this area like DIP receives output from GP, but unlike DIP, it projects to the rostral Wulst, an area that gives rise to descending projections very similar to the pyramidal tract in mammals (Wild 1992, Wild 1997, Wild and Williams 1999, Wild and Williams 2000). Obviously if rostral Wulst is in fact a motor cortex like region in birds, then it would make sense to conclude that the GP-->VIA-->Wulst pathway is a homolog of the GP-->VA/VL->motor cortex pathway in mammals, and this would indirectly support the idea that the dorsal thalamic nuclei are homologous to the intralaminar and midline nuclei. In a separate section below I outline the anatomical evidence that the Wulst is a motor cortical like region involved in a network similar to the pyramidal tract. In a follow up study Veenman et al. (1997) used immnohistochemical and hodological evidence to argue that DT is similar to the intralaminar midline complex in mammals. They present evidence that DT, similar to the mammalian intralaminar nuclei, stains more heavily for GABA, substance P, and CHAT than the surrounding sensory relay nuclei. They also provide evidence from tracer injection studies that DT projects upon the striatum with a topography similar to that seen for the intralaminar nuclei. I note that the CHAT stain in DT, while stronger than in areas immediately ventral to it, is quite light, unlike the stain for AChE in the intralaminar nuclei (Steriade, Pare et al. 1988). The tracer studies, although in agreement with the Wild's investigation of a possible avian thalamostriatal system (1987), did not include any studies with injections in DT that would show anterogradely labeled terminals of thalamic neurons in striatum.

Recall from the section on the intralaminar nuclei in mammals that the main afferents that distinguish them from motor thalamus are from the superior colliculus and from acetylcholinergic

and other cell groups in the brianstem. In birds the equivalent structure to the superior colliculus is the optic tectum, a structure with neocortex-like lamina that occupies the optic lobe, the grossly obvious outgrowth from the midbrain in birds and fish. With reference to the cerebellar-recipient part of dorsal thalamus in particular, two groups have reported that injections in DLP result in retrograde label of neurons in optic tectum(Gamlin and Cohen 1986, Korzeniewska and Güntürkün 1990) and this input has been confirmed with injections in optic tectum (Wylie, Glover et al. 1998). Although Arends and Zeigler argued that CbN output targeted and optic tectum target distinct subregions of DLP, no study has tested this directly with multiple tracer injections, and as far as I can tell no similar study has been carried out in mammals to test to what extent input from the superior colliculus and cerebellum overlap in CL and the other intralaminar nuclei. At the very least these anatomical findings in birds suggest the inputs of DT are similar to the inputs of the intralaminar nuclei. Similarly, multisensory responses have been reported in DLP (Korzeniewska and Güntürkün 1990) as has been reported for the intralaminar nuclei, where these responses are thought to be mediated by deeper layers of the superior colliculus (Smith, Galvan et al. 2014). As for evidence that cholinergic groups project to DT, there has not been as far as I can tell any retrograde tracer studies carried out in birds similar to those that showed in mammals that the intralaminar nuclei preferentially receive input from brainstem acetylcholinergic neurons. One study of ChAT in the pigeon brain did identify DT as a region where there were many fibers immunoreactive for ChAT (Medina and Reiner 1994) but there also many perikarya in the same regions that were positive for signal from the same antibodies, and these are a logical source for those fibers. Two studies of ChAT and Ache have been done in zebra finches; while both report heavy stain of perikarya and fibers in basal ganglia song system nucleus Area X, one makes no

mention of thalamic song system nucleus DLM (Zuschratter and Scheich 1990), and the other specifically states it was indistinguishable from the surrounding DT (Sadananda 2004) which was lightly stained.

One last afferent to DT that might provide insight into whether it is like ventral motor thalamus or the intralaminar nucleus is ascending somatosensory projections from the dorsal column nuclei. In mammals, these nuclei principally relay somatosensory information from the spine. They target ventral thalamus, specifically the region that projects to somatosensory cortex, and so are thought of as the "sensory" region of ventral thalamus. Tracer studies show that the targets of the dorsal column nuclei in thalamus do not include the intralaminar nuclei, and their projections to ventral thalamus are segregated from the projections of the cerebellar nuclei (Asanuma, Thach et al. 1983, Aumann, Rawson et al. 1996). In pigeons (Wild 1989) and in songbirds (Wild 1997), the dorsal column nuclei project to several nuclei in dorsal thalamus, but they mainly target the so-called dorsal intermediate ventral area (DIVA). Similarly to the target of the dorsal column nuclei in mammalian ventral thalamus, VPL, the nucleus DIVA relays somatosensory input to a cortical region in birds that is the source of a putative avian pyramidal tract, a tract I describe in some detail below. As noted in discussion of these results (Wild 1989), the connections of DIVA clearly make it a close cousin to VPL in mammals, and this provides evidence—again circumstantial—that dorsal thalamus in birds might actually be more like ventral motor thalamus in mammals. Again I emphasize it is important to consider this "circumstantial evidence" because of the reported differences between thalamostriatal projections originating from the intralaminar nuclei and those that originate from the rest of mammalian thalamus.

As for evidence of a thalamostriatal projection specifically in songbirds, a handful of studies report retrograde label of cell bodies of DT when making tracer injections in the basal ganglia nucleus of the song system, Area X. Lewis et al. (1981) provided some of the first evidence that VTA is the source of dopaminergic input to Area X. They also report retrograde label of neurons in DIP from injections of horseradish peroxidase in Area X. This result was consistent across animals, but they also saw similar label in DIP after a control injection in striatum posterior to X. They do not methodically map the label or even show images of the retrograde label in DIP, but they do state that injections in nidopallium dorsal to Area X did not retrogradely label cells in DIP. This is a bit surprising given that nidopallium dorsal to the medial striatum in pigeons is the target of DIP in pigeons (Kitt and Brauth 1982) and surrounding nidopallium is targeted by neighboring dorsal thalamic regions (Gamlin and Cohen 1986, Wild 1987). Castelino et al. (2007) made large pressure injections of cholera toxin B in Area X with the purpose of identifying its noradrenergic inputs. They like Lewis et al. noted retrograde label of cell bodies in dorsal thalamus, in regions they identified as DLL and DLP. Another study reported similar label from smaller iontophoretic injections of cholera toxin B in Area X (Person, Gale et al. 2008). But as the authors of that study and others have recognized, these results are confounded by the possibility of tracer uptake by fibers of passage *en route* to cortex from thalamus. Specifically, song system nucleus DLM projects to LMAN, and its axons traverse Area X on their way to the nidopallium directly dorsal of Area X where LMAN is found (Bottjer, Halsema et al. 1989).

I.2.C Open question about the song system: is there a route through thalamus from the cerebellar nuclei to the song system?

As mentioned above, there has been only one extensive study of the projections of CbN in birds (Arends and Zeigler 1991), carried out in pigeons. Previous work in songbirds provided some evidence that in these species the CbN project to dorsal thalamus. One study of the known song system nuclei in thalamus noted retrograde label in CbN after tracer injections in DT (Vates, Vicario et al. 1997). The previously cited extensive mapping of the songbird basal ganglia (Person, Gale et al. 2008) specifically tested whether there might be a disynaptic pathway from CbN to Area X through dorsal thalamus. As noted in the preceding section, the authors showed that iontophoretic injections of CtB in Area X retrogradely labeled neurons in regions of dorsal thalamus, and in the same animals showed that injections of dextran amines in CbN anterogradely labeled terminals in the same regions. But the authors noted the passing fibers confound described above, and also emphasized that they had not directly demonstrated the existence of thalamostriatal axon terminals. To do so would require anterogradely labeling the terminals. This was done at the light microscopic levels in the first studies to demonstrate thalamostriatal terminals in mammals using tracers in place of earlier degeneration methods. Later work confirmed the projections formed synapses in striatum with electron microscopy.

Taken as a whole, these anatomical and electrophysiological results suggest there is no clear one-to-one correspondence between avian and mammalian thalamic nuclei involved in motor control. They also demonstrate the need to use other methods to demonstrate whether there is a thalamostriatal projection in songbirds. As I describe below, I will combine lentiviral and standard

tracers to determine whether there is a thalamostriatal projection and a cerebellothalamic route into the song system.

I.2.D Non-classical components of the song system

Finally, before describing the specific hypotheses about neuroanatomy tested by this dissertation, I will briefly describe some findings about pathways outside of the classical song system. These findings raise two important questions. (1) At what points does the song system interact with other systems in the brain, and in particular other motor systems? It is convenient to think of the song system as isolated from the rest of the brain, but in fact a songbird sings with the same beak it uses to eat and with the same lungs it uses to breathe. (2) Did the song system evolve from motor pathways shared by all vertebrates, and could a similar process of evolution have given rise to speech in humans?

I.2.D.1 Descending motor pathways in the avian brain

When first introducing the mammalian motor system, I described a hierarchical model in which motor cortical areas sat at the top. These motor cortical areas project to the spinal cord, in some cases directly to motor neurons. The same motor cortical regions also characteristically project to the red nucleus, the pontine nuclei, the inferior olive, and sensory nuclei in the brainstem as well as brainstem regions that contain interneurons that modulate the activity of motor neurons in the cranial nerve. Many of the nodes in this network in turn send projections to cerebellar cortex and receive projections from the cerebellar nuclei. Through these pathways both cortex and cerebellum can modulate activity of motor neurons, including those that are involved with vocalization. As I

stated at the beginning of the section on motor systems, in songbirds the output of the song system must at some point interact with the output of other motor systems that control muscles used in multiple contexts, for example the jaw muscles that open the beak for singing and for eating. In the Discussion I propose where interactions between the song system and other motor systems might take place. So that I can do so, I now outline evidence for a pathway in the avian brain with similarities to the pyramidal tract.

This pathway originates from the Wulst (German for "bump"), a region found at the very anterior end of the avian brain with a laminar structure. It is widely considered the most likely candidate for a homolog to mammalian neocortex (Karten and Shimizu 1989). The Wulst can be divided into two parts, a caudal region involved mainly with visual pathways, and a rostral region that gives rise to the pyramidal tract-like descending projections. The tract specifically arises from a layer named accessory hyperpallium (HA). As cited above, HA is the major target of nucleus DIVA in dorsal thalamus that relays somatosensory input from the dorsal column nuclei. Like the pyramidal tract in mammals (Kuypers 1981), the projections of HA have been shown in both pigeons (Wild 1992) and songbirds (Wild and Williams 2000) to target the red nucleus (Humphrey, Gold et al. 1984), the pontine nuclei (Kelly and Strick 2003), and the inferior olive (Sedgwick and Williams 1967). In addition, like the pyramidal tract, HA projects to the spinal cord, decussating at the level of the hindbrain. There its targets include the motor neurons that control neck muscles (Wild and Williams 2000), although a direct projection to spinal motor neurons from cortex is usually thought to be a feature seen only in primates. Furthermore HA like mammalian motor cortex projects to

the dorsal column nuclei that relay ascending somatosensory information (Wild and Williams 2000).

Of particular relevance to the questions addressed in this dissertation is how the cerebellum interacts with several nuclei that are also targets of the descending projections of HA. A schematic of the relevant pathways is shown in Figure 13. In addition to its projections to the red nucleus and pontine nuclei, HA has been shown to project to SpM, a pre-cerebellar nucleus identified in birds and fish (Karten and Finger 1976) that sends mossy-fiber like projections with large terminals to lobules VI through IX of the cerebellum (Clarke 1977, Wild 1992). In pigeons, it has been shown that in addition to input from SpM, lobule VI also receives input from the red nucleus (Wild 1992). Other authors have not noted retrograde label in the red nucleus after injecting cholera toxin B in lobule VI, but they have shown that this lobule is the target of several nuclei involved in visual processing (along with lobules VII and VIII)

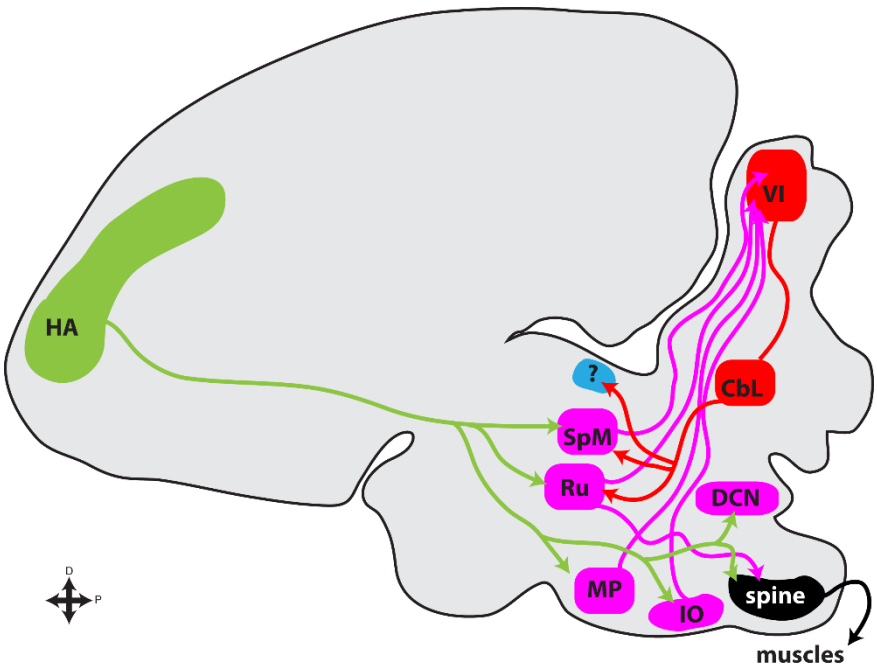

Figure 13. A pyramidal tract in the songbird involves the lateral cerebellar nucleus. HA, a cortex-like region at the rostral pole of the songbird brain, projects to many pre-cerebellar nuclei, similar to the pyramidal tract in mammals. These targets include the red nucleus (Ru), the inferior olive (IO), and the dorsal column nuclei (DCN), and the spinal cord. Many of these nuclei project to the cerebellum, as does the medial spiriform nucleus (SpM), a pre-cerebellar nucleus found in birds that is also a target of HA. In addition CbL likely projects to some part of dorsal thalamus (indicated with "?")

(Pakan and Wylie 2006). Those authors did find that injections in the same regions of lobule VI retrogradely labeled SpM. Lateral regions of lobule VI then project to the lateral cerebellar nucleus, CbL (as do lateral regions of almost all lobules) (Arends and Zeigler 1991). Similar to the cerebellar nuclei in mammlas, CbL sends a major projection to the red nucleus, and in addition it projects to SpM (Wild 1992) as well as the principle precomissural nucleus (PPC), that also receives input from HA and projects to the pontine nuclei (not shown in figure). Furthermore the red nucleus projects upon the dorsal column nuclei and the spinal cord, again as it does in mammals (Wild 1992). Based on its connections, then, the lateral CbN would appear to be not just a potential pathway for cerebellar output to reach the song system via dorsal thalamus, but also a key node in the motor system of birds. This then raises the question of whether separate population of neurons in lateral CbN project to dorsal thalamus and the red nucleus or if, as in mammals, individual CbN axons send collaterals to the red nucleus on their way to dorsal thalamus, providing copies of the same output to both regions. As described above, there is evidence that many of the brainstem motor nuclei involved in phonation are targets of the red nucleus in mammals. An early degeneration study of the red nuclei in pigeons specifically noted a lack of evidence for it projecting to the facial motor nucleus (Wild, Cabot et al. 1979). A study of the trigeminal and facial motor nucleus in pigeons also did not report retrograde label of the red nucleus; it did report such label in the cerebellar nuclei but conclude it arose due to uptake by passing fibers (Berkhoudt, Klein et al. 1982). A study of the trigeminal motor nuclei in zebra finches also did not report any retrograde label of the red nucleus (Wild and Krützfeldt 2012). Likewise, studies of nucleus retroambigualis and the hypoglossal nucleus in songbirds have also not reported any retrograde label of the red nucleus or cerebellum (Wild, Kubke et al. 2009), unlike what was reported for

primates. Finally a recent study of the sensory trigeminal nuclei did in some cases report anterograde label in the cerebellum from tracer injections, but some of this label was due to traversing the cerebellum to inject in the nuclei (Faunes and Wild 2017). However the same study did show that thalamic somatosensory nucleus DIVA, a major source of input to HA, does itself receive input from the sensory regions of the trigeminal nucleus in songbirds (Faunes and Wild 2017). So there is evidence that ascending sensory information from the jaw enters this pyramidal tract-like system, but the weight of the anatomical evidence suggests there are limited or no monosynaptic connections between the cerebellum and the brainstem nuclei involved in vocalization, or even possible interactions via the red nucleus and those brainstem nuclei.

Returning to HA, in addition to its pyramidal tract-like projections just described, it also projects to areas in the forebrain which are interesting because of their relation to the song system (Wild and Williams 1999). Injections in HA yield anterograde label of fibers that surround and sometimes invade several song system nuclei: in particular, Area X and LMAN. As the authors state, these regions are very likely the same regions that have been proposed as a parallel loop through the anterior forebrain pathway, the so-called “shell” pathway. In this pathway, a “shell” area ventral and medial of DLM in thalamus projects to a shell outside LMAN that then projects to arcopallium outside of RA (Johnson, Sablan et al. 1995), a region I will refer to as intermediate arcopallium (Ai). A follow-up study (Bottjer, Brady et al. 2000) of the projections of Ai found that it projected to many areas not classically considered part of the song system: for example, pre-cerebellar nucleus SpM. One behavioral study found that lesioning Ai did not have immediate effects during development but prevented song from “crystallizing” (Bottjer and Altenau 2010).

In contrast, another study investigating a nearby region of arcopallium found during the course of control experiments that large lesions of Ai resulted in severe akinesia and immobility that, obviously, prevented birds from singing (Mandelblat-Cerf, Las et al. 2014). It is very possible that one or both groups lesioned an area in arcopallium that is specialized for control of the beak. Anatomical studies show that this beak area in arcopallium does project to SpM, and is the main target of a distinct forebrain nucleus that receives ascending information from avian brainstem regions similar to the sensory trigeminal nucleus (Wild and Farabaugh 1996). It may even be the case that the groups carrying out behavioral studies and the anatomists are all discussing the same region, but this is obscured because of how results are typically presented. In any case, the anatomical studies and behavioral results taken together highlight the need to understand where and how the output of the song system and other motor systems in the avian brain take place. Previous authors have observed that the connections of the AFP in the song system parallel connections between striatum, thalamus, and the two cortical regions nidopallium and arcopallium in non-songbird species, and that this could be the ancestral motor pathway in birds (Farries 2001). I emphasize that a major target of the outflow of arcopallium, including Ai, is SpM, the pre-cerebellar nucleus, in spite of the lack of evidence, reviewed above, that cerebellar output could reach brainstem motor nuclei that are ultimately responsible for vocalization.

I.2.D.2 Other connections

Other studies have also pointed towards the same question of how the song system interacts with the rest of the brain. For example, the output neurons of Area X that project to DLM also send collaterals to ventral pallidum (VP), an area that is found in all bird brains (not just songbirds) and

does not contain a discrete song system nucleus. VP then projects to arcopallium outside of RA, and this part of arcopallium, known as the AIV (Intermediate ventral arcopallium) projects to VTA, specifically to neurons that project back to Area X (Gale, Person et al. 2008). In addition, AIV receives descending projections from high-level auditory areas. *In vivo* extracellular recordings from neurons in AIV suggest they show responses to auditory feedback, and lesions of AIV impair song learning in juveniles (Mandelblat-Cerf, Las et al. 2014). (This is the same study that made lesions of Ai as a control.)

I.2.D.3 Hypothesized evolutionary origins of neural pathways for vocal behavior

Briefly I mention a hypothesis that has been proposed for how the neural system evolved that supports learned vocalizations. This hypothesis holds that the song system evolved in part from motor systems common to all vertebrate brains (Jarvis 2004). Others have similarly observed that there appears to be a general motor pathway in the avian brain from which the song system might evolve (Farries 2001). Support for this hypothesis comes in part on comparison of immediate early gene (IEG) expression resulting from singing and from moving in songbirds (Feenders, Liedvogel et al. 2008). Singing resulted in heavy expression of the mRNA of the IEG Zenk (EGR-1 in mammals) within the song system. Moving resulted in heavy expression of Zenk mRNA in areas outside the song system, particularly the "shell" areas that surround the nuclei just described above. Several genomic and bioinformatics studies have further supported the idea that song system nuclei are close in gene expression to the shell regions that surround them.

I.3 Overview of studies in this dissertation

As stated in the Introduction and discussed at length in the preceding review, two unanswered questions about the song system are:

(1) Is there a thalamostriatal projection, i.e. a projection from some part of thalamus to the basal ganglia nucleus, Area X?

(2) Is there a path from the cerebellar nuclei through thalamus to song system nuclei in the forebrain?

I address these questions with neuroanatomical studies in Bengalese finches. This songbird species that is of interest because it depends strongly on auditory feedback to maintain its song in adulthood (Okanoya and Yamaguchi 1997, Woolley and Rubel 1997) and has been shown to adapt its song to perturbations of auditory feedback (Sober and Brainard 2009, Sober and Brainard 2012) in a manner reminiscent of cerebellar-dependent sensorimotor adaptation. To answer the questions outlined, I use standard neuroanatomical tracers and lentiviral vectors. The main experiments are schematized in Figure 14. I first demonstrate with dextran amine injections that the cerebellar nuclei project to dorsal thalamus in the Bengalese finch. Then I present evidence for a thalamostriatal projections to the song system obtained with lentiviral vectors. Because these viral vectors are only taken up by the cell body at the injection site, they exclusively provide anterograde label. In addition, one of the vectors I use encodes synaptophysin tagged with green fluorescent

protein (GFP), so that it preferentially labels presynaptic axon terminal segments. By combining lentiviral vectors with immunohistochemistry, I demonstrate that Area X does receive thalamic input. My results suggest that Area X receives input from both DLM, the thalamic song system nucleus, and an adjacent subregion of cerebellar-recipient dorsal thalamus. I also present results obtained with standard tracers that show cerebellar output is unlikely to reach other song system nuclei through dorsal thalamus, although it may reach cortical regions outside the song system. In addition, I present other results from injections in the cerebellar nuclei and in globus pallidus that are relevant to questions raised in the preceding literature review about motor systems in birds.

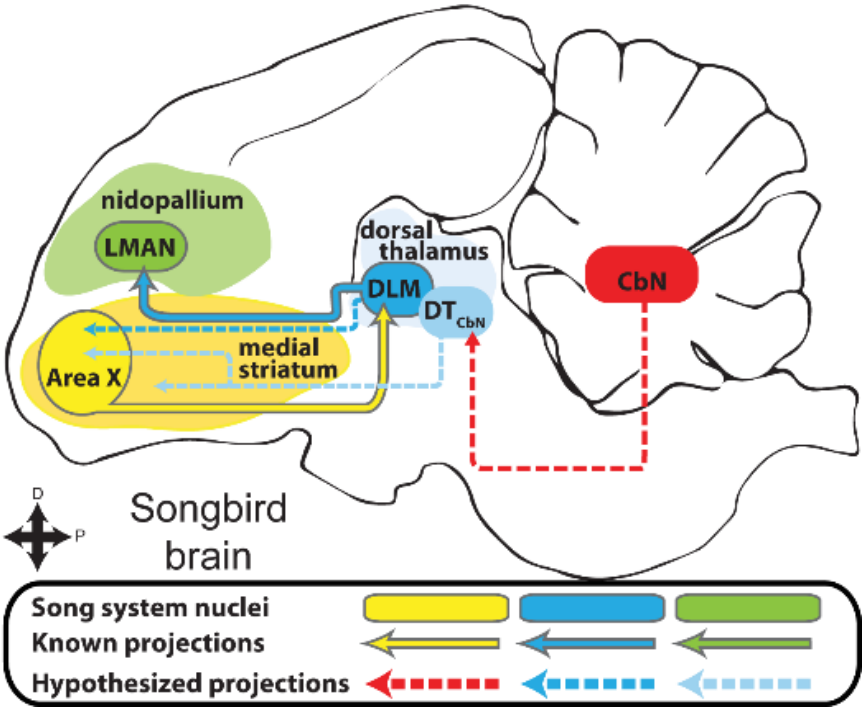

Figure 14. Outline of main experiments. The anterior forebrain pathway (AFP) is a thalamocortical-basal ganglia loop in the song system required for learning song. It consists of basal ganglia nucleus Area X (yellow oval), thalamic nucleus DLM (blue oval) and cortical nucleus LMAN (green oval, known song system connections outlined in gray. The rest of the song system is not shown.). Using lentiviral tracing methods, I test whether thalamus projects to the basal ganglia in a songbird, the Bengalese Finch. Specifically I test whether Area X (yellow oval) receives input from DLM (darker blue dashed arrow). I also tested whether Area X or the surrounding medial striatum receive input from cerebellar-recipient dorsal thalamus (DTCbN, lighter blue dashed arrows). In order to identify targets of DTCbN, I first confirmed that the cerebellar nuclei (CbN) project to dorsal thalamus.

II Methods

All studies were carried out in adult (>90 days post hatch) male Bengalese finches either bought from a supplier or bred in our laboratory. Age of purchased birds was assessed by screening them for adult-like song. Work reported here was approved by the Emory University Institutional Animal Care and Use Committee.

II.1 Surgery and tissue collection.

Neuroanatomical tracers and lentiviral vectors were injected with a stereotaxic apparatus (Leica/MyNeuroLab), using the co-ordinates in Table 1 to target brain regions described in the text. For all surgeries, anesthesia was induced with ketamine-midazolam and when necessary supplemented with isoflurane (0.25-2.5%). After induction, the bird's scalp was anesthetized locally with ~20uL lidocaine. An incision was made and the top layer of the skull was removed to localize the landmark "Y0". Y0 was defined as the most posterior point visible at the junction of the midsagittal sinus and the two sinuses that run on either side of the cerebellum. After moving the pipette to the target co-ordinates on the surface of the skull, a craniotomy was made, the dura was opened with a syringe needle, and then the pipette was lowered to the target depth. Iontophoretic injections were made with an A&M 2100 stimulator, 7 seconds on, 7 seconds off, 4-10 µA, positive current, with a total injection time of 20-30 minutes. Iontophoresis was used to inject fluorophore-tagged dextran amines, 10% in 0.1M phosphate buffer, and in some cases cholera toxin B, 2% in 0.1M phosphate buffer. Pressure injections were made with a Nanoject II (Drummond). Lentiviral vectors were a 1:1 solution of mCherry and synapthophysin tagged with

GFP. mCherry labeled cell bodies and axons, whereas synaptophysin-GFP specifically labeled synapses. These vectors were developed for anterograde tracing in songbirds; for details see (Bauer, Coleman et al. 2008, Roberts, Klein et al. 2008). Typical injection parameters for lentiviral vector injections with the Nanoject were 32.2 nl per press of the "inject" button, at a speed of 23 nl/second, for a total of 800-1500 nl in dorsal thalamus. Typically there was a 45 second pause between each press of the "inject" button, and after injecting the entire volume the pipette was left undisturbed for 5m, then raised slowly (~100 μm every 5 seconds).

After surgery, birds survived 5-7d when standard tracers were used, and 20-30d when lentiviral vectors were used. After the appropriate survival time, birds were sacrificed with an overdose of ketamine and midazolam, supplemented with isoflurane when necessary. The perfusate consisted of ~20mL 0.95% saline with heparin, followed by ~50mL 4% paraformaldehyde in 0.1M phosphate buffer (PB). The brain was removed and left in 4% paraformaldehyde, 30% sucrose solution overnight at room temperature, then transferred to 30% sucrose in 0.1M PB, where it was left until it sank in solution. Brains were cut parasagitally or coronally on a sliding freezing microtome and 30-60um sections were stored in 0.1M PB for further processing.

Table 1. Co-ordinates for stereotaxic surgery.

Target	**Anterior of y0**	**Lateral of y0**	**Depth**	**Beak bar angle below horizontal**
CbL	-1.2 – -1.6	1.3 – 1.4	3.25-3.45	50
Medial dorsal thalamus	0.9-1.1	1.3	4.1-4.3	45
Area X	5.5-5.7	0.9-2.2	2.9-3.1	20

II.2 Immunohistochemistry

Sections were rinsed for ten minutes in 0.1M PB, then washed in 2% sodium borohydride in 0.1M PB for 0.5h, as a form of epitope retrieval. This was followed by three more washes for 10m in 0.1M PB. Then sections placed for 1h in a block solution of 2.5% normal donkey serum, 2.5% normal horse serum, 1% Triton-X 100 in 0.1M PB. Primary antibodies were diluted in 1% NDS, 1% NHS, 0.3% TX-100. Sections were incubated 24-48h at 4°C in primary solutions. Then the sections were rinsed 3x10m in 0.1M PB before incubating with secondary antibodies that were either tagged with fluorophores or biotinylated. Both secondary and tertiary solutions consisted of 0.3% TX-100 in 0.1M PB, and incubations in these solutions lasted 1h. In cases where a tertiary was used (e.g., streptavidin-AMCA), the incubation was preceded by 3x10m washes in 0.1m PB. All tertiaries used were streptavidin conjugates, and were used at a 1:200 concentration. Tertiaries were Vector labs streptavidin-AMCA (SA-5008) and Thermo Fischer streptavidin-Alexa Fluor 633 (S21375). In all cases, sections were washed 3 more times for 10m in 0.1M PB before they were mounted on Fischer Superplus slides, then left overnight to dry. Sections were briefly

rehydrated before using Fluoro-Gel with DABCO mounting medium to apply coverslips, and then sealing coverslips with clear coat fingernail polish.

Initial experiments used Rockland rabbit anti-RFP and Invitrogen mouse anti-GFP antibodies to amplify mCherry and GFP-synaptophysin expression. In later experiments where Millipore mouse anti-Parvalbumin was used as a marker for Area X and other song system nuclei, mCherry signal was amplified with Invitrogen rat anti-mCherry and synaptophysin-GFP signal was amplified with Invitrogen rabbit anti-GFP.

II.3 Production and specificity of antisera

Please see Table 2 for list of antibodies used.

Name	Immunogen structure	Manufacturer, catalog #, RRID, species, mono/poly	Concentration
Anti-red fluorescent protein	mushroom polyp coral Discosoma	Rockland 600-401-379, AB_2209751, Rabbit polyclonal	1:1000
Anti-mCherry	full-length protein mCherry	Life technologies/Invitrogen M11217, AB_2536611, Rat monoclonal	1:1000
GFP tag (clone 3E6)	GFP isolated directly from Aequorea victoria	Life technologies/Invitrogen A-11120, AB_2534132, Mouse monoclonal	1:2000
GFP tag	GFP isolated directly from Aequorea victoria	Life technologies/Invitrogen A-11122, AB_2534134, Rabbit polyclonal	1:2000
Parvalbumin	Parvalbumin purified from frog muscle	EMD Millipore MAB1572, AB_2174013, Mouse monoclonal	1:2000
Rhodamine Red X donkey anti rabbit	whole molecule rabbit IgG	Jackson 711-295-152, AB_2340613, donkey polyclonal	1:400
Rhodamine Red X donkey anti rat	whole molecule rat IgG	Jackson 712-295-150, AB_2340675, donkey polyclonal	1:400
Alexa 488 donkey anti-mouse	whole molecule mouse IgG	Jackson 715-545-150, AB_2340846, donkey polyclonal	1:400
Alexa 488 donkey anti-rabbit	whole molecule rabbit IgG	Jackson 711-545-152, AB_2313584, donkey polyclonal	1:400
Biotinylated horse anti-mouse IgG (H+L)	whole molecule mouse IgG	Vector laboratories BA-2000, AB_2313581, horse, polyclonal	1:400

Table 2. Antibodies used.

Anti-red fluorescent protein (RFP) antibody, Rockland 600-401-379, AB_2209751, Rabbit polyclonal, detects RFP but not GFP as shown by Western blot (manufacturer's datasheet). The antibody has been used previously to amplify mCherry signal expression from viral vectors (De

Arcangelis, Liu et al. 2009, Dinh and Bernhardt 2011) including vectors used in neuroscience studies (Redondo, Kim et al. 2014, Sreenivasan, Karmakar et al. 2015).

Anti-mCherry, Life technologies/Invitrogen M11217, Rat monoclonal, specifically detects mCherry as shown with Western blot and flow cytometry (manufacturer's datasheet). The antibody has been used previously in virally-mediated neural tracing experiments (Schwarz, Miyamichi et al. 2015).

Anti-GFP, Life technologies/Invitrogen A-11120, mouse monoclonal, was raised against GFP isolated from Aequorea Victoria (manufacturer's datasheet). It has been shown to detect GFP fusion proteins expressed in neurons under genetic control (Busch, Selcho et al. 2009, Liu, Luo et al. 2015) and GFP expression resulting from viral vectors (Keen-Rhinehart, Michopoulos et al. 2009, Vujovic, Gooley et al. 2015)

Anti-GFP, Life technologies/Invitrogen A-11122, rabbit polyclonal, was raised against GFP isolated from Aequorea Victoria (manufacturer's datasheet). Previous reports show this antibody detects GFP expression induced in neurons by viral vectors (Davis, Choi et al. 2011, Lindberg, Chen et al. 2013)

Anti-parvalbumin, EMD Millipore MAB1572, mouse monoclonal, was raised against parvalbumin purified from frog muscle, and in Western blots yields a band at 12kDa (the weight of parvalbumin). The antibody shows specific immunoreactivity with parvalbumin expressing interneurons (McKenna, Yang et al. 2013) and has been used to label such neurons in songbirds (Li, Zhou et al. 2013). It has previously been shown that one cell type in Area X expresses

parvalbumin and that Area X shows higher immunoreactivity for parvalbumin in its neuropil than the surrounding medial striatum (Braun, Scheich et al. 1985, Carrillo and Doupe 2004, Reiner, Laverghetta et al. 2004)

II.4 Microscopy, digital photography, and image processing.

Low-power widefield images were obtained with a Zeiss Axioplan 2 and Olympus IX51. Confocal z-stacks were obtained with Leica SP8 inverted and Olympus FV1000 inverted microscopes. Brightness and contrast were adjusted using ImageJ, Zeiss AxioVision software (for images acquired with the Axioplan 2), or Photoshop (for images acquired with the Olympus IX51). ImageJ was used for all processing of z-stacks, including z projections, adjustment of brightness and threshold, and changing of look-up tables (e.g., to convert red to magenta). The following procedure was used to calculate the distances from the midline shown in figures on parasagittal sections: the zero point was chosen to be the middle of the interstitial nucleus of Cajal (InC), which is found at the midline and which usually occurred in two consecutive sections when the brain was cut parasagittaly; then the number of sections was counted including the section in the figure and the section of InC between that section and the zero point; that number of sections was multiplied by the section thickness (e.g. 20 sections x 40 micrometers/section) and lastly the total number of micrometers was multiplied by 1.5, a factor to account for shrinkage that occurred when the brain was fixed. This factor was obtained by measuring the distance from the midline to injection sites in fixed tissue, solving algebraically for the factor that when multiplied by the fixed distance resulted in the distance used during surgery, and averaging that factor across birds.

II.5 “Drawings” of signal from lentiviral injections.

To present results from lentiviral injections, a procedure was followed that yielded figures similar to camera lucida-assisted drawings from light microscopy material. After performing fluorescence immunohistochemistry on sections to amplify signal, large tiled scans were made of the sections with the confocal microscopes using a 40x objective. Then z-projections were made of these tiled scans, compressing the z-stack into one x-y plane, and brightness and contrast were adjusted in ImageJ. In Adobe Illustrator, the tiled z-projection of each section was aligned with a widefield image taken with a 4x objective of the same section. The 4x objective was used with a DAPI filter to image the Parvalbumin (PV) signal, to identify the borders of Area X and other areas of interest. To ensure that the 40x images and the 4x images were at the same scale, scale bars of the same size were placed on both images and aligned before aligning the images themselves. The 40x images were then made partially transparent in Illustrator and aligned to the 4x sections by eye using landmarks, e.g., the edges of the sections and cytoarchitectural landmarks that were visible because of slight background autofluorescence. Using a stylus with a Wacom graphics tablet, regions of interest such as Area X were outlined based on the PV signal. Next the aligned images and outlined regions were imported into Adobe Photoshop. On the layers with the 40x tiled z-projections, the Lasso tool was used to outline all areas of signal (either synaptophysin-GFP imaged with Alexa 488 secondaries or mCherry imaged with Rhodamine Red X). These areas with signal were copied to a separate layer and then the Invert and Threshold functions were applied to that layer. This converted the signal to black regions. The Photoshop file with signal converted to black regions was opened in Illustrator, and the Image Trace tool was applied to the inverted and

thresholded signal (mode: black and white, threshold: 210-245), paths: 100%, corners: 75%, noise: 15-25px, create: fills, snap curves to lines: no, ignore white: yes). This created a "tracing" of the signal. Lastly the "Make and Expand" function was applied to convert the Image Trace objects to vector art, and the vector art was colored to indicate the type of signal (magenta for mCherry, green for synaptophysin, gray for various levels of Parvalbumin intensity). Finally this completed tracing was exported as a .png file.

II.6 Map of dorsal thalamus

To produce a reference map of dorsal thalamus, regions of interest in one bird were outlined where GFP expressing viral vector was injected in Area X to label its projection to DLM, and dextran amines were injected in CbL to label DT_{CbN}. Only one bird was used as a reference to be sure there was no added noise due to slight differences in alignment when sectioning brains and then aligning series of section by eye. Hence in this reference series it was clear where DLM and DT_{CbN} were located in each section relative to each other. As shown in Figure 16, projections of CbL were consistent across animals. Likewise when aligning this reference map with series from birds with injections only in Area X, DLM as defined by its input from Area X was consistent across animals (Appendix V.3,Figure 31). In addition DLM could be recognized even in unstained tissue because of the heavier myelination that showed up as a brighter area when sections were viewed with darkfield through a DIC filter of a 5x objective.

Injection sites from birds with viral injections in dorsal thalamus were aligned with the map of its regions using cytoarchitectural landmarks that were clearly visible when viewing unstained

sections at 5x with darkfield. (DIC was combined with darkfield because this increased contrast on the microscope used to image injection sites, a widefield Zeiss Axioplan 2). Typical injection sites consisted of neurons expressing either synaptophysin-GFP or mCherry, with a sparse population of cells expressing both. Injection sites were defined by mCherry labeled cell bodies because the label was easier to image on the widefield at 5x. Comparison of this label with synapthophysin-GFP label in the injection site as imaged with a confocal showed no obvious difference between the injection site as defined by mCherry signal or synaptophysin-GFP signal—i.e. injection sites appeared to be mostly homogenous mixture of neurons infected with one or the other viral vector.

III Results

III.1 The cerebellar nuclei in Bengalese Finches project to dorsal thalamus

These studies focus on two possible sources of thalamic projections to Area X: song system thalamic nucleus DLM and the area of dorsal thalamus that surrounds it and is thought to receive output from the cerebellar nuclei. I refer to this area as cerebellar-recipient dorsal thalamus, DT_{CbN}. By identifying DT_{CbN} in Bengalese finches, I could determine whether cerebellar output could reach the basal ganglia, possibly including Area X, through DT_{CbN}, similar to cerebello-thalamic-striatal pathways in rats and primates (Ichinohe, Mori et al. 2000, Hoshi, Tremblay et al. 2005, Chen, Fremont et al. 2014)

III.1.A CbL projects to dorsal thalamus

As described in the Introduction, the finding that the cerebellar nuclei project to dorsal thalamus and that these projections surround but do not invade thalamic song system nucleus DLM was previously shown in zebra finches (Person, Gale et al. 2008). For that reason I first confirmed that the projection from CbN to contralateral dorsal thalamus occurs in Bengalese finches. The initial set of injections targeted the lateral cerebellar nuclei (CbL) because of previous work showing this was the main source of projections to thalamus. Injections of dextran amines in Bengalese finch CbL yielded anterograde label across the mediolateral extent of dorsal thalamus. Figure 15 presents a series of sections showing the anterograde label across dorsal thalamus from a representative case. This result was consistent across animals (n=3, Figure 16). In every case where I successfully targeted CbL, I saw label across the entire mediolateral extent of dorsal

thalamus, from near the midline (Figure 15L, 0.78 mm lateral) to very laterally near the pretectal nucleus (Pt) (Figure 15C, 2.12 mm lateral). I always saw a densely labeled area posterior to songbird DLM (Figure 16C, black arrows with white outline), in roughly the same mediolateral plane as the auditory region of thalamus, nucleus ovoidalis (NOv). This area of dense label extended laterally to the same mediolateral plane as the retinal-recipient nucleus DLL (Figure 16D, white arrows), posterior to the region that receives retinal output as shown in an atlas of the zebra finch brain (Karten, Brzozowska-Prechtl et al. 2013). It

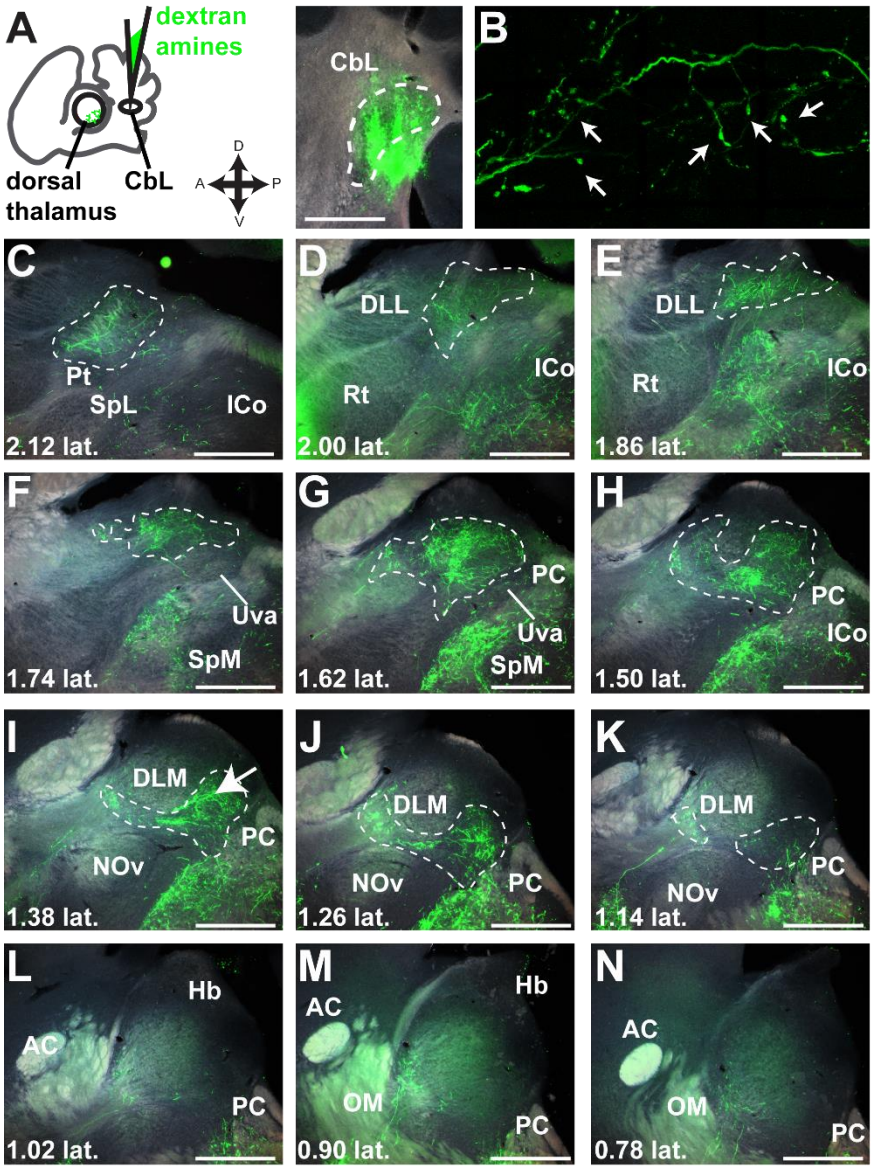

Figure 15. CbL projects to contralateral dorsal thalamus. **A,** Schematic (left) of experiment showing injection in CbL and widefield image (right) showing injection site. **B,** High-resolution confocal image of axon-terminal like morphology in dorsal thalamus. This image was taken from the area indicated with a white arrow in **I**. Panels c-n are the representative series of widefield images across dorsal thalamus showing anterograde label from the injection in CbL. **C** is the most lateral section and **N** is the most medial. Dotted white lines demarcate the areas considered cerebellar-recipient dorsal thalamus. Left is anterior and up is dorsal. This series is from "bird 3" in Figure 5. All scale bars 500 µm.

should be noted that in addition to the label in dorsal thalamus, I also saw widespread signal throughout the midbrain, with dense innervation of the red nucleus, ansa lenticularis, and inferior colliculus, consistent with what was reported by Person et al. (2008) and what is seen in other birds and mammals (Medina, Veenman et al. 1997, Hoshi, Tremblay et al. 2005). These other afferents are described in more detail in the Appendix (section V.2.C).

Some injections in the cerebellar nuclei yielded some retrograde label of the medial spiriform nucleus (SpM), usually with stronger signal ipsilateral to the injection site. Injections that missed CbN also yielded retrograde label of SpM, so it is not clear if this retrograde label results from a projection to CbN or is due to tracer uptake by passing fibers. SpM is known to project to the cerebellum (Karten and Finger 1976, Person, Gale et al. 2008) and in particular to the cerebellar cortex (Wild 1992). It is possible that some of the label in dorsal thalamus was actually collaterals from SpM, because I saw such collaterals ipsilaterally (i.e., it appeared that the tracer traveled retrogradely from the cerebellum to SpM cell bodies and then from SpM traveled anterogradely to label collaterals in dorsal thalamus). However, there were few of these collaterals and they appeared qualitatively different from the dense label seen contralaterally, in that the putative SpM collaterals had long branching segments near the terminals. (For further discussion see Appendix V.2.B and Figure 26) I also examined results from dextran amine injections in dorsal thalamus (those in Figure 17 and Figure 18) for evidence of retrograde label in SpM. In some cases there were sections with 1-2 retrogradely labeled cell bodies in SpM, but this could also have resulted from tracer that spread outside the targeted area. Therefore the majority of label in contralateral dorsal thalamus traveled anterograde from CbL.

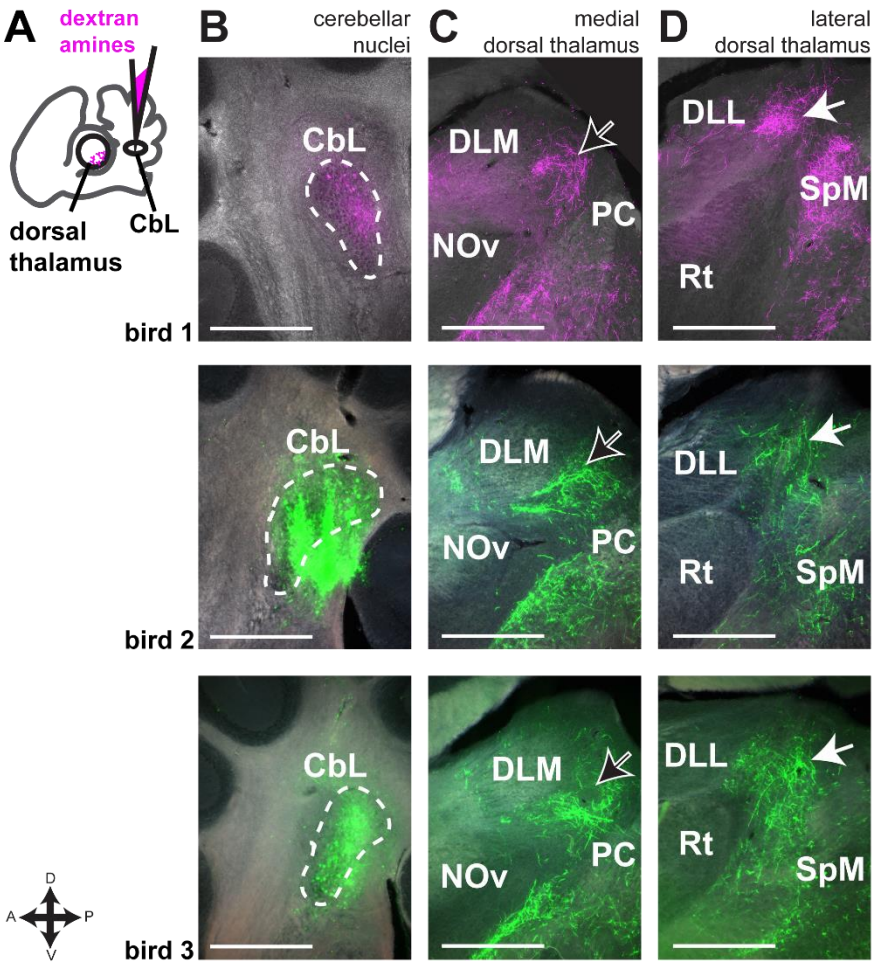

Figure 16. CbL axon terminals target medial dorsal thalamus adjacent to song system nucleus DLM, but also target more lateral dorsal thalamus. **A,** Schematic representation of experiment 2, showing injection site in lateral cerebellar nuclei, and anterograde label in contralateral dorsal thalamus. **B,** Injection sites. **C,** More medial site in dorsal thalamus with strong label. See for comparison Person et al 2008, figure 19. **D,** More lateral site in dorsal thalamus with strong label. All sections are parasagittal, left is anterior and up is dorsal. All scale bars 500 µm.

III.1.B CbI and CbM also project to dorsal thalamus

To confirm that CbL projects to dorsal thalamus, I made injections of dextran amines in dorsal thalamus and looked for retrograde label in CbL (Figure 17A). These injections yielded retrograde label of contralateral CbN (Figure 17B, C). As previously reported in songbirds (Vates, Vicario et al. 1997), the injections gave strong label in CbL. In addition, the injections yielded retrograde label in intermediate and medial regions of the cerebellar nuclei (CbI and CbM, Figure 17D), consistent with what has been reported for other birds (Medina, Veenman et al. 1997) and for mammals (Hoshi, Tremblay et al. 2005). I combined results from animals (n=3) in which the mediolateral position of the thalamic injection site varied (Figure 18) to map of the regions of cerebellar nuclei that project to dorsal thalamus in a songbird. In two of these birds I made multiple injections in the mediolateral plane of dorsal thalamus (Figure 18, injection sites colored cyan and magenta), which yielded strong retrograde label. A more ventral and medial injection (Figure 18, injection site colored yellow) yielded less retrograde label. All three injections yielded the strongest retrograde label in CbL, but they also yielded label of many neurons in CbI and a few neurons in CbM as well (Figure 18B). Hence, results suggest that the strongest projection to dorsal thalamus originates from CbL, but that there is a significant contribution from CbI, and some input from CbM as well.

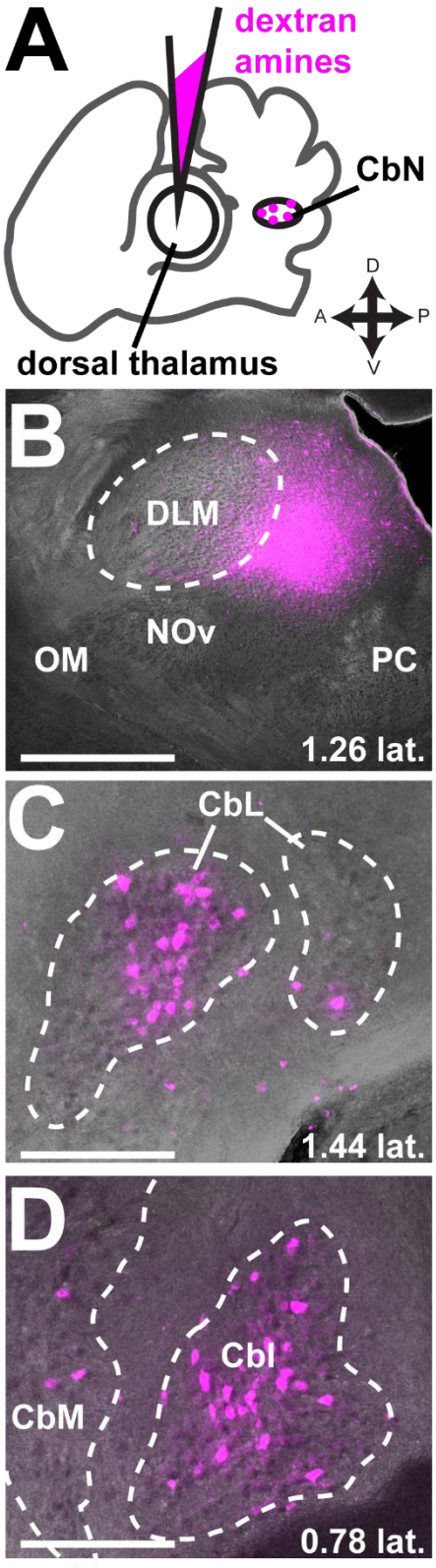

Figure 17. Projections from all of CbN to dorsal thalamus. **A,** schematic indicating injection site in dorsal thalamus and site of retrograde label in contralateral CbN (shown in same cartoon "section"). **B,** Representative injection site in dorsal thalamus. Parasagittal section. Anterior is left and dorsal is up. **C,** Retrograde label in contralateral CbL (lateral cerebellar nuclei). **D,** Retrograde label in contralateral CbI and CbM (intermediate and medial cerebellar nuclei). Scale bar, 500 µm.

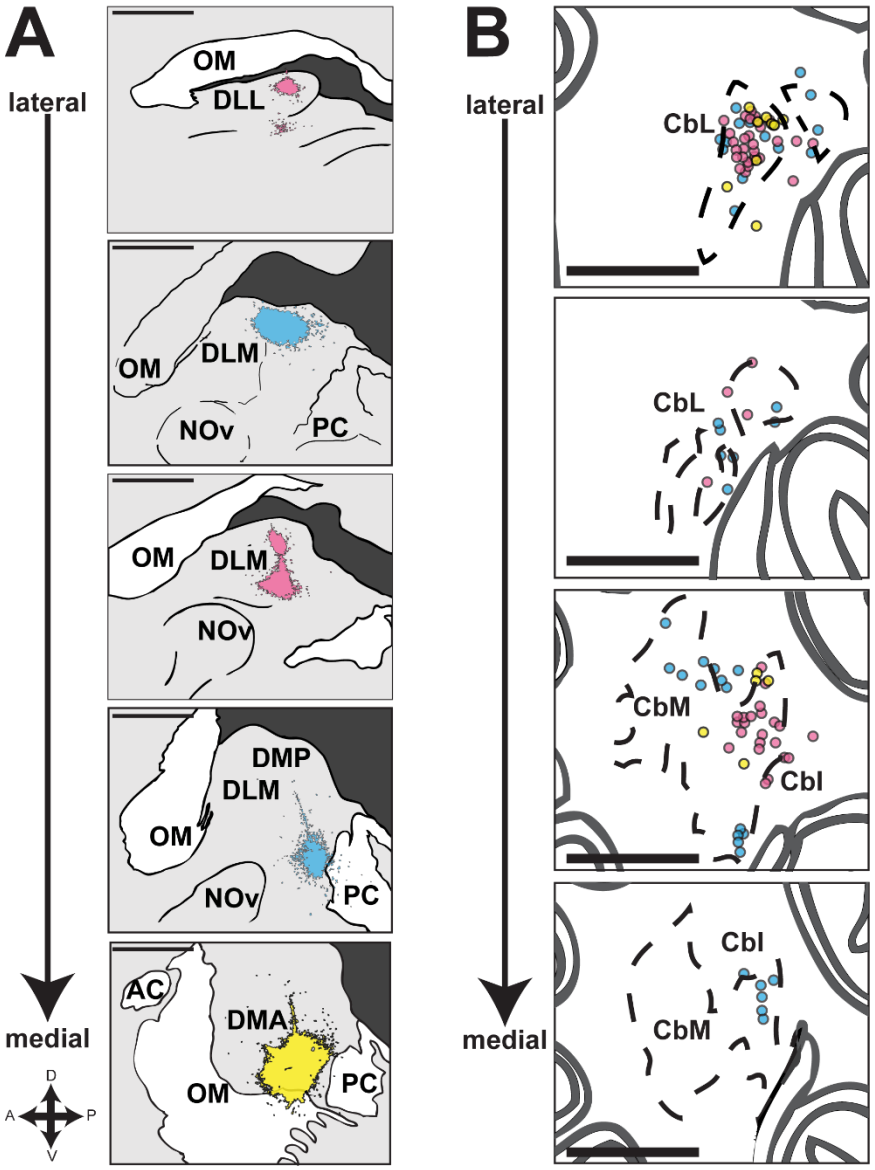

Figure 18. All of the cerebellar nuclei project to dorsal thalamus. **A,** Injection sites in dorsal thalamus from 3 birds (magenta, cyan, yellow) arranged from lateral to medial. Note that the 3rd panel from the top corresponds approximately to the injection site shown in figure 17B. **B,** Schematic of retrograde label in the contralateral cerebellar nuclei arranged from lateral to medial. All scale bars 500 µm

III.2 Dorsal thalamus in Bengalese Finches projects to Area X

It has not been shown previously whether the thalamus projects to the basal ganglia in songbirds, as occurs in mammals. To determine whether songbirds have a thalamostriatal system, I used lentiviral vectors for neuroanatomical tracing. This made it possible to circumvent confounds associated with standard tracers. For example, standard tracers travel to some extent in both the anterograde and retrograde direction (Kobbert, Apps et al. 2000, Reiner, Veenman et al. 2000), whereas the lentivirus used infects only cell bodies at the injection site, so that any label seen outside the injection site is from anterograde transport (Grinevich, Brecht et al. 2005, Roberts, Klein et al. 2008). In addition, one lentiviral vector used encodes GFP-tagged synaptophysin. Because synaptophysin is transported to presynaptic axon terminals, due to its protein targeting signals, the virus specifically labels presynaptic axon segments with GFP signal (Grinevich, Brecht et al. 2005, Roberts, Klein et al. 2008). I made injections in dorsal thalamus (experiment schematic, Figure 19A) containing a 1:1 ratio of the synaptophysin-GFP vector and another expressing mCherry (Roberts, Klein et al. 2008). The mCherry vector labels cell bodies and axons, facilitating the identification of the injection site in dorsal thalamus (Figure 19B) and follow the path of axons. In no case did viral injections produce retrograde label (e.g., label of cell bodies in Area X, medial striatum, or CbL) from injections in dorsal thalamus. Thus I am confident that results are based exclusively on signal produced by infection of cell bodies local to the injection site and transported in the anterograde direction.

Injections in dorsal thalamus yielded synaptophysin-GFP label in medial striatum (Figure 19D, E). The axon segments labeled by synaptophysin-GFP had numerous varicosities (Figure

19E), suggesting they formed synapses *en passant*, as seen in mammalian thalamostriatal systems (Berendse and Groenewegen 1990, Deschenes, Bourassa et al. 1996). In all animals where viral injections were made in dorsal thalamus, Area X was labeled by performing immunohistochemistry against parvalbumin (PV) (Figure 19C). Neuropil in Area X is more strongly enriched in PV than the surrounding medial striatum. This made it possible to clearly identify Area X in each section and determine whether processes labeled by synaptophysin-GFP and mCherry were within its

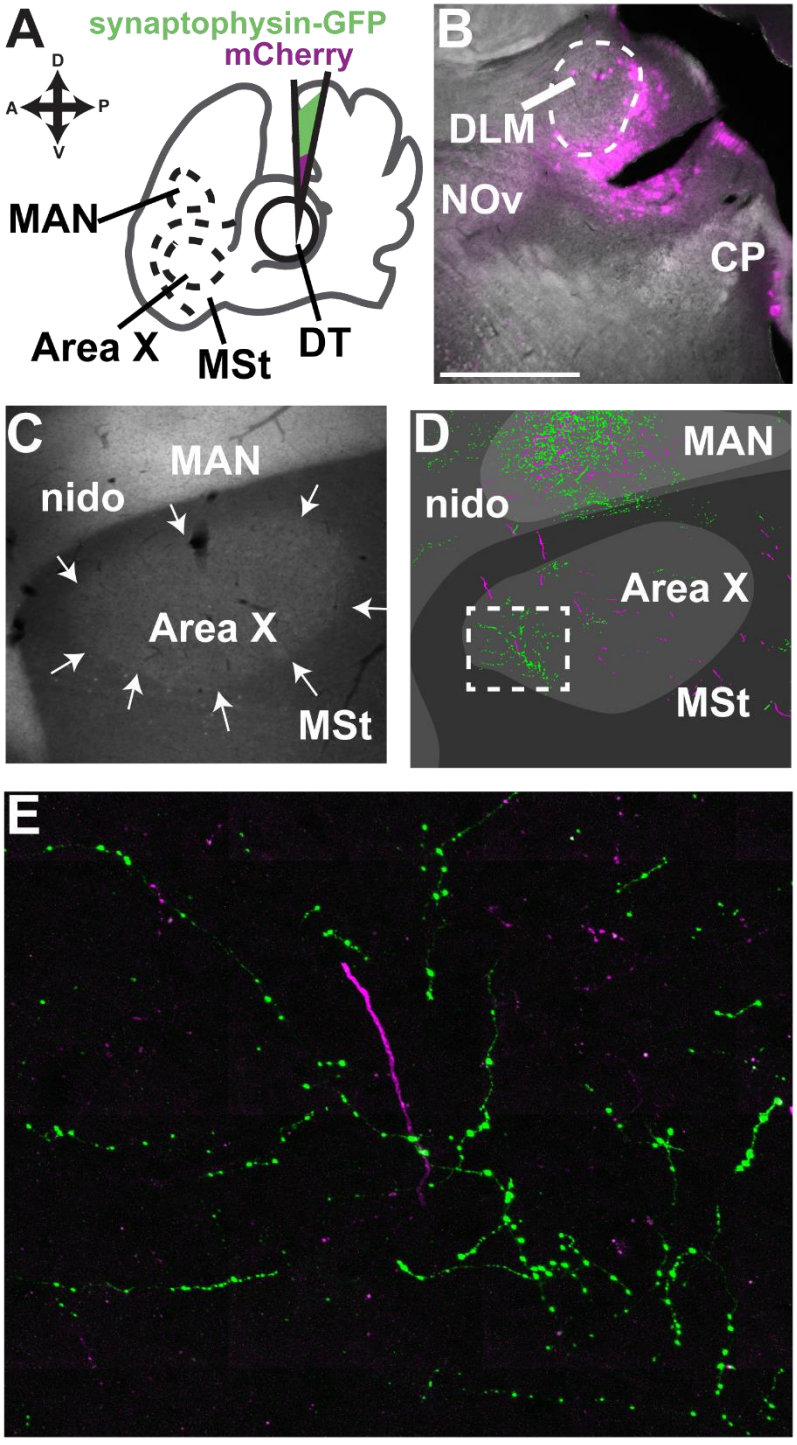

Figure 19. Dorsal thalamus projects to striatum, including Area X. **A,** Schematic of experiment. A 1:1 solution of two lentiviral vectorsone expressing synaptophysin-GFP and the other mCherry was injected into dorsal thalamus. **B,** Widefield image of injection site from representative bird. **C,** Parvalbumin stain to outline Area X (white arrows) from the same bird. **D,** "Camera lucida" style tracing of GFP and mCherry signal from same section. **E,** Confocal image of area shown in white box in **D**. Note varicosities suggesting *en passant* synapses. Scale bars: **A-D,** 500 µm; **E,** 250 µm.

borders. Aligning PV-stained section with mosaic images of high-powered confocal stacks imaging the GFP and mCherry revealed that dorsal thalamus projected to Area X (Figure 19D).

Initial injections were large and included DLM and surrounding dorsal thalamus. In addition, the anatomy of dorsal thalamus has not been extensively studied in songbirds, so it was not clear what regions outside of DLM might have been included in the injections. For these reasons I decided to delineate regions of dorsal thalamus in terms of their inputs and outputs.

III.2.A DLM and the immediately adjacent DT_{CbN} project to Area X

Having identified the regions of dorsal thalamus that receive cerebellar output, DT_{CbN}, I then determined whether this subregion, or DLM, or both subregions project to Area X. I made a series of viral vector injections in dorsal thalamus and for each I aligned the injection site with a reference map of DLM and DT_{CbN} (described in Methods). In addition to mapping regions of interest in dorsal thalamus, I made smaller injections in some birds so the injection site would be contained to one region of thalamus. I found that whenever the injection site included DLM or the immediately adjacent DT_{CbN} (n=3 hemispheres from two birds, Figure 20A,E,I), it produced anterograde label in Area X (Figure 20B-D,F-H,J-L). In two of the three cases, there was strong label of processes with varicosities, similar to what is shown in Figure 19E, across Area X (Figure 20B and K, arrow with white outline). In the remaining case, there was less label in Area X (Figure 20F, arrow with white outline) and there was also label of processes with varicosities just ventral to Area X (Figure 20F, solid white arrow). There was no obvious topography in the dorsoventral

or anteroposterior planes, although often the label was strongest in the same mediolateral plane as the injection site. Because of space constraints, the entire series from each case is not shown here, but can be seen in the supplementary information at https://doi.org/10.6084/m9.figshare.5437975.

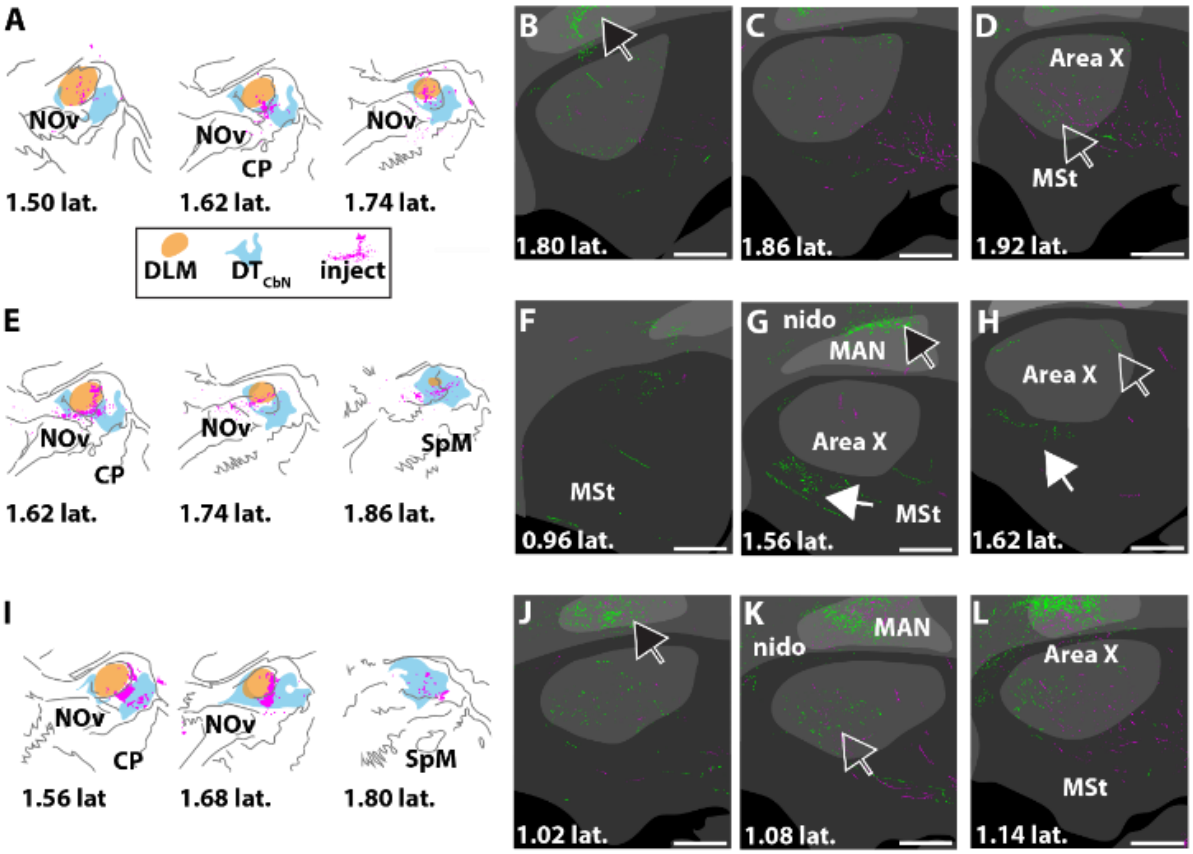

Figure 20. DLM and adjacent DTCbN project to Area X. **A-D,** case where injection was mostly in DLM. Synaptophysin-GFP label was evident in Area X, arrow with white outline in **D**. **E-H,** case where injection was in DLM and surrounding DT_{CbN}. Synaptophysin-GFP label was evident in Area X, arrow with white outline in **H**, and in medial striatum, white arrow in **G** and **H**. **I-L,** case where injection was mostly in $DT_{CbN.}$ Although the injection was mainly in DT_{CbN}, it again produced strong synaptophysin-GFP label in Area X, arrow with white outline in **K**. **A,E,I,** Injection sites. Orange region, DLM; cyan region, DT_{CbN}; magenta, cell bodies expressing mCherry. **B-D, F-H, J-L.** Series of sections from lateral to medial showing Area X, surrounding medial striatum, and overlying nidopallium. Green, GFP signal. Magenta, mCherry signal. Dark gray, no parvalbumin label; gray, some parvalbumin label; light gray, strong parvalbumin label. Arrow with white outline, GFP signal in Area X; solid white arrow, GFP signal outside Area X in MSt; black arrow with white outline, GFP signal in nidopallium. All scale bars 500 µm.

III.2.B More medial and ventral DT_{CbN} projects to medial striatum outside of Area X

Because of the difficulty of targeting small regions of dorsal thalamus with the large volume of solution required for viral vector injections, I was not able to successfully target just DLM or just the adjacent DT_{CbN}. However I can distinguish the results from these injections (Figure 20) from

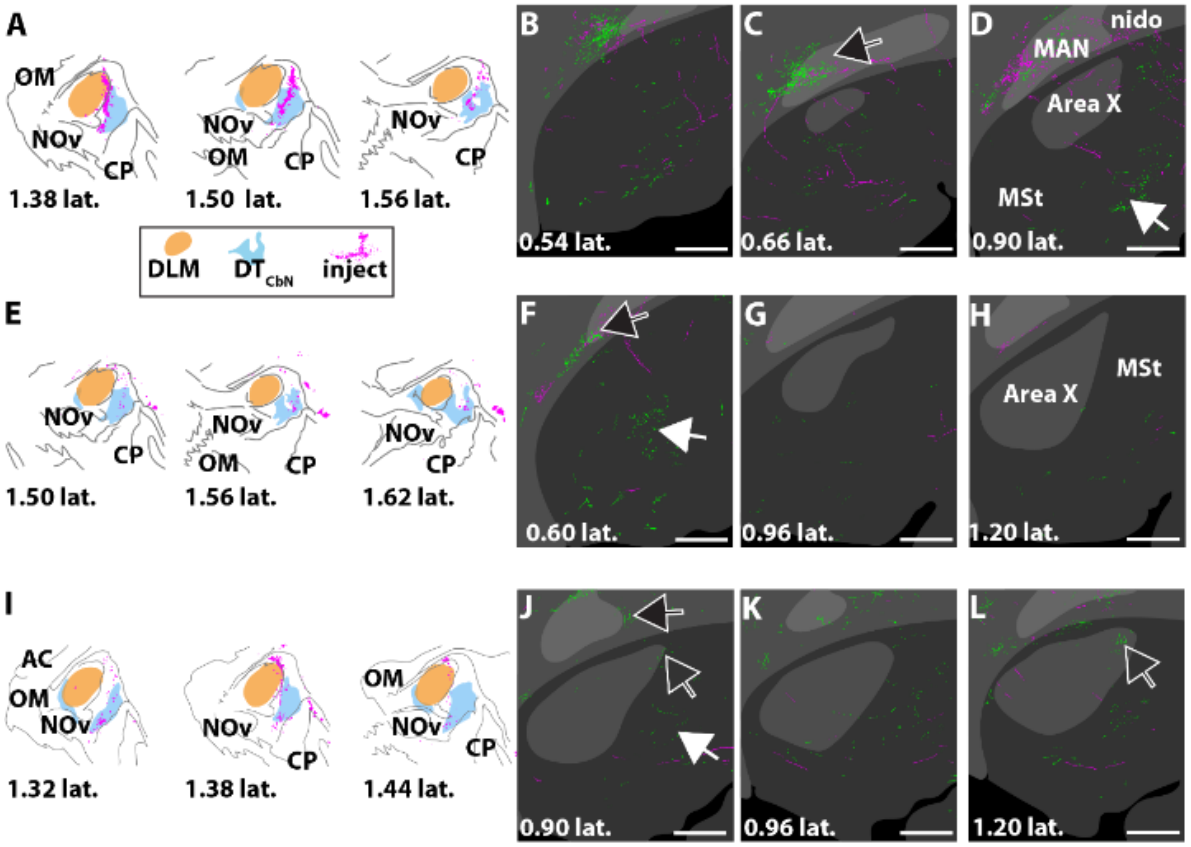

Figure 21. More medial and posterior regions of DT_{CbN} DLM project to medial striatum. **A-D,** case where injection was mostly in DT_{CbN} and more medial. Strong synaptophysin-GFP label was posterior and/or medial of Area X, for example see panel D, white arrow. There was almost no GFP label in Area X in this case. **E-H,** case where injection was in DT_{CbN} but posterior of DLM. Again the strongest Synaptophysin-GFP label was medial of Area X—e.g., panel F, white arrow. **I-L,** in this case there was a small amount of label in Area X, arrow with white outline in J and L, but the majority of the label was again in medial posterior striatum, white arrow in panel J. **A,E,I,** Injection sites in DLM and DT_{CbN} for 3 birds. Sections are arranged from medial to lateral reading from left to right. Orange region, DLM; cyan region, DT_{CbN}; magenta, cell bodies expressing mCherry. **B-D, F-H, J-L,** Series of sections from lateral to medial showing Area X, surrounding medial striatum, and overlying nidopallium. Green, GFP signal. Magenta, mCherry signal. Dark gray, no parvalbumin label; gray, some parvalbumin label; light gray, strong parvalbumin label. . Arrow with white outline, GFP signal in Area X; solid white arrow, GFP signal outside Area X in MSt; black arrow with white outline, GFP signal in nidopallium. All scale bars 500 µm.

those of another set of injections that were confined to the more medial and posterior portion of DT_{CbN} separate from DLM (n=3, Figure 21A,E,I). The results from these three birds suggest that this more medial and posterior portion of DT_{CbN} does not target Area X. Instead it projects to medial striatum posterior and ventral Area X (Figure 21B,H,L, solid white arrows).Occasionally there were small areas of GFP-signal within Area X (Figure 21L, arrow with white outline) from these injections, but strongly-labeled processes with varicosities were posterior and ventral to Area X, and they were much more medial (see for example Figure 21H and L). Label was usually strongest in the same mediolateral plane as the injection site. Again because of space constraints, the entire series from each case is not shown here, but can be seen in the supplementary information at https://doi.org/10.6084/m9.figshare.5437975.

Note also that regardless of injection site I saw strong label of synaptophysin-GFP and mCherry processes in the cortical layer overlaying the basal ganglia known as the nidopallium. I could not clearly differentiate song system nucleus LMAN from the surrounding nidopallium based on parvalbumin labeling in the same way that I could differentiate Area X from medial striatum. However, it was clear that injections that included DLM produced label in LMAN as expected (for example Figure 20D,G, and L, black arrow with white outline), and that injections in DT_{CbN} separate from DLM tended to produce label in nidopallium outside of LMAN but still within an area of somewhat stronger labeling for parvalbumin (Figure 21C, H, and L, black arrow with white outline). These results suggest that either individual neurons in dorsal thalamus project to both striatum and cortex, or that thalamic neurons that project to striatum are intermingled with those that project to cortex.

IV Discussion

Results from anatomical studies in Bengalese finches addressed two questions about the song system: whether it contains a thalamostriatal projection, and whether it receives input from the cerebellum. Figure 22 summarizes the results. As shown with dextran amine injections, the cerebellar nuclei project to contralateral dorsal thalamus (Figure 15,Figure 16,Figure 17,Figure 18); as above, this cerebellar-recipient region will be referred to in the Discussion as DT_{CbN}. Lentiviral vectors were used to show that dorsal thalamus does project to Area X, the basal ganglia nucleus of the song system, as well as the surrounding medial striatum (Figure 19).

. By mapping viral injection sites in relation to DT_{CbN} and neighboring thalamic song system nucleus DLM, it was shown that DLM and the subregion of DT_{CbN} immediately adjacent to it project to Area X, and that

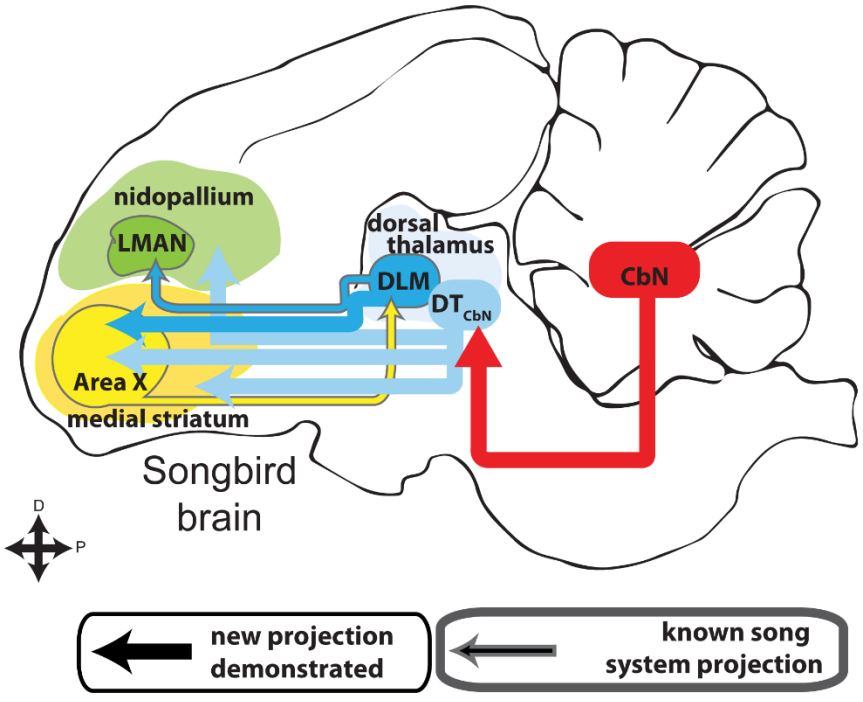

Figure 22. Schematic summarizing principal findings. New projections demonstrated by results above are shown as thick arrows. Results demonstrated that the cerebellar nuclei project to the contralateral dorsal thalamus (red arrow) in Bengalese finches, the region I refer to as DT_{CbN}. In turn, DT_{CbN} projects to the basal ganglia nucleus of the song system, Area X, as well as the surrounding medial striatum and to nidopallium (lighter blue arrows). In the song system, thalamic nucleus DLM also projects to Area X (darker blue arrow).

the more medial and ventral subregion of DT_{CbN} projects to medial striatum posterior and ventral to Area X (Figure 20, Figure 21). Below, I discuss the strengths and weaknesses of these results, provide context from previous work, and also propose follow-up studies and discuss the functional implications.

IV.1 Technical considerations, and relation to previous work

IV.1.A Cerebellothalamic projections

The results from the dextran amine injections in CbL showed that these nuclei project to a broad mediolateral swath of dorsal thalamus. Interestingly, anterograde label from injections in CbL seemed to avoid song system thalamic nucleus DLM (see for example Figure 15 and Figure 16), although there were heavily-labeled regions posterior and anterior of it. The same injections yielded anterograde label in many sites throughout the midbrain, and in some cases retrograde label of SpM, a thalamic nucleus that projects to the cerebellum, and as noted, some label in dorsal thalamus might be due to "retrograde" label of collaterals from SpM (Karten and Finger 1976) (Appendix V.2.B, Figure 26). These collaterals were sparse though. These results from injections in CbL are consistent with those of Person et al. (2008), the only previous study that has considered anterograde label from CbN injections in a songbird. Retrograde label in CbL after tracer injections in DT_{CbN} confirmed that CbL projects to dorsal thalamus. These results were also consistent with previous work in songbirds (Vates, Vicario et al. 1997). Retrograde label was also seen in CbI and CbM after tracer injections in dorsal thalamus (Figure 17, Figure 18). This suggests all regions of CbN project to dorsal thalamus, a finding that as far as I know has not been previously been reported for songbirds. However, many studies in pigeons that report retrograde label of the

cerebellar nuclei from injections in dorsal thalamic nuclei (Wild 1988, Korzeniewska and Güntürkün 1990, Wylie, Glover et al. 1998), and Medina et al. specifically reported retrograde label of CbI and CbM from dorsal thalamus tracer injections in pigeons (Medina, Veenman et al. 1997).

Injections of dextran amines in CbL also demonstrated that the cerebellum projects to many other sites throughout midbrain and hindbrain. Specifically CbL target the red nucleus (Appendix V.2.C, Figure 27), a nucleus that may have a reciprocal projection to cerebellar cortex. The red nucleus also receives input from motor cortex-like region HA (Wild 1992), and these cortical and cerebellar connections form part of a pyramidal tract-like network in the songbird brain. In addition CbL projects to SpM (Appendix V.2.C, Figure 29), the pre-cerebellar nucleus found in birds and fish, and the principal pre-commisural nucleus, another region whose targets include the pontine nuclei. HA has also been shown to project to SpM and PPC, and in addition HA projects to the pontine nuclei and the inferior olive, two regions intimately involved with cerebellar function (Wild 1989, Wild 1997, Wild and Williams 1999, Wild and Williams 2000). SpM clearly targets lobule VI (as well as VII and VIII) and the red nucleus has also been reported to project to lobule VI. Intriguingly, the strongest retrograde label of Purkinje cells after injections in CbL was in lobule VI (Appendix V.2.D, Figure 30). Experiments to determine how these other projections of CbL relate to its projection to dorsal thalamus are outlined below in the section on follow-up studies (sections IV.2.B).

IV.1.B Thalamostriatal projections

Although prior anatomical studies have suggested that the thalamus projects to the striatum in birds, as it does in mammals (Lewis, Ryan et al. 1981, Wild 1987, Veenman, Karle et al. 1995, Castelino, Diekamp et al. 2007, Person, Gale et al. 2008), several methodological confounds have prevented a definitive demonstration that this projection occurs in birds. These confounds have been recognized previously (Bottjer, Halsema et al. 1989, Person, Gale et al. 2008). Briefly, retrograde label is not sufficient to demonstrate such a projection because of a fibers of passage confound that arises due to DLM axons passing through Area X to arrive at LMAN. Standard tracers injected into dorsal thalamus and then analyzed at the light microscopic level also do not provide strong evidence of a projection from thalamus to Area X, because retrograde label of the projection to thalamus from Area X will also result from standard tracers. It can be hard to differentiate anterograde and retrograde label at the light microscopic level, and there is the possibility that what appears to be anterograde label is just a retrogradely labeled axon or collateral of a neuron whose cell body is in an adjacent section of tissue. To circumvent these problems, I used lentiviral vectors, which infected the cell body yielding signal that only traveled in the anterograde direction (Grinevich, Brecht et al. 2005, Bauer, Coleman et al. 2008, Roberts, Klein et al. 2008). Using a vector encoding synaptophysin tagged with GFP made it possible to specifically label presynaptic terminals. Injections of this vector in dorsal thalamus resulted in significant synaptophysin-GFP label in Area X and MSt outside Area X (Figure 19, Figure 20, Figure 21). This finding provides strong evidence for a thalamostriatal system in songbirds, although I acknowledge it is not as conclusive as, for example, a demonstration of synapses with

electron microscopy (which is challenging in the songbird brain compared to e.g. primates because of the difficulty of sectioning tissue on a vibratome). I emphasize the following findings that strengthen the argument: the synaptophysin-GFP labeled processes had varicosities suggestive of *en passant* synapses, as reported for the thalamostriatal system in mammals (Berendse and Groenewegen 1990, Deschenes, Bourassa et al. 1996), and this morphology was noticeably different from that of other axons passing through Area X on their way to LMAN and the surrounding nidopallium. It could be possible that ectopic expression of synaptophysin due to the viral infection somehow results in creation of these synapses, but there were also axons labeled only by mCherry that had varicosities and local processes in the striatum. Therefore the varicosities and collaterals of axons in the striatum I observed are likely to represent the true morphology of these processes. By the same token, some thalamic injections yielded very strong synaptophysin-GFP label in the motor cortex-like region HA (presumably because the injection site included thalamic nucleus DIVA that has been previously shown to target HA). Axons labeled by these injections passed through medial striatum en route to anterior forebrain, but there were no synaptophysin-GFP labeled varicosities in Area X or medial striatum in these animals (Appendix V.4.E, Figure 37).

A "map" was produced of dorsal thalamus, so that injection sites could be projected onto this map. In this way it could be determined what regions when injected with virus yielded label of axon terminals in Area X. The map defined DLM as the region that received Area X output as shown with injections of dextran amines and with a GFP-encoding AAV vector in Area X (Appendix V.3, Figure 31). In the same way, DT_{CbN} was defined as the region that showed anterograde label after

dextran amine injections in CbL, as described in the main results (Figure 15). That region was used as a proxy for "DT_{CbN}", although the rest of the cerebellar nuclei send some projections to dorsal thalamus as well (Figure 17, Figure 18). I did investigate the topography of CbL and CbI projections to dorsal thalamus, and in one experiment successfully targeted CbI with fluorescein-tagged amines and CbL with rhodamine-tagged amines. Results from this one case suggest there may be subtle topography in the projections of CbN to DT (Appendix V.2.A, Figure 25), but it appears in general the regions labeled after injections of CbL serve as a good approximation of "cerebellar-recipient dorsal thalamus". Injections were also made in globus pallidus to define DIP, because previous studies in pigeons identified this pallidal-recipient area in dorsal thalamus and suggested that it projected to the striatum and globus pallidus. Injections in globus pallidus demonstrated that DIP lies ventral to DLM and anterior of DT_{CbN} (Appendix V.3, Figure 32). DIP is too small to target with large injections required for lentiviral vector, but when injection sites included DIP, the results were similar to injections in posterior medial DT_{CbN} (Figure 21). This finding is consistent with other previous tracing studies and does not address which regions target Area X and medial striatum, so DIP was not included in the map of dorsal thalamus used to obtain the results in the main study.

Results obtained by imposing injections sites on the map of dorsal thalamus showed that, surprisingly, both DLM and immediately-adjacent DT_{CbN} specifically target Area X (Figure 20). Importantly, this finding cannot be completely explained by spill of viral vector from DLM into DT_{CbN} or vice versa: there were cases with strong synaptophysin-GFP label in Area X when the injection was mostly contained to DLM (Figure 20E) and also when it was mostly contained to

DT_{CbN} (Figure 20I). The results are also not an artefact of how injection sites were defined. Injection sites in dorsal thalamus as defined by mCherry signal were not noticeably different from sites defined by synaptophysin-GFP signal (which was not strong enough in thalamus to image at low power with the widefield microscope used to document injection sites, but was obvious when imaged with a confocal).

IV.1.C Disynaptic pathway from CbN to Area X through DT_{CbN}

One approach that future studies could use to determine whether CbN axon terminals directly synapse with thalamic neurons would be to pair tracer injections in putative thalamic targets in the forebrain with injections in CbN. In the case of Area X, results would be unclear, because of the confounds described above: there is the passing fibers issue where DLM axons pass through Area X on their way to LMAN, and there is the issue that LMAN sits directly above Area X and so many routes for injecting tracer in Area X could result in spill into LMAN. Still if there is a disynaptic pathway from CbN to Area X through DT_{CbN} as suggested by lentiviral injections in thalamus, one would expect to find positive evidence of it from paired injections of retrograde tracer in Area X and anterograde tracer in CbN. Such injections did yield occurrences of anterogradely-labeled CbL axon terminals closely apposed to dendrites and soma of retrogradely-labeled thalamic neurons (Appendix V.4, Figure 33). However there were few of these occurrences. In part this could be because thalamic neurons were often lightly labeled from the injections of CtB in Area X even though known afferents of Area X such as HVC, VTA, and LMAN showed moderate to dense label. The light label and the low number of occurrences could both be explained in part by the number and nature of thalamostriatal synapses: compared to the

dense terminals seen in LMAN and nidopallium from viral thalamic injections, the processes in Area X and medial striatum were qualitatively sparse and the varicosities appeared to be *en passant* synapses with few locally branching terminals.

Although the results provide evidence for one route from the cerebellar nuclei to a song system nucleus through dorsal thalamus, there are other such routes that could be hypothesized, and it could be the case that DT_{CbN} projects to regions outside the song system. Two other potential routes through thalamus to song system nuclei would be through DMP to MMAN or through Uva to HVC. Paired tracer injections did not provide evidence that CbL output could reach the song system via these hypothesized pathways (Appendix V.4, Figure 34, Figure 35). The strongest evidence for a disynaptic route from CbL to the forebrain outside the song system was found when paired injections were made in CbL and in nidopallium just rostral to LMAN (Appendix V.4, Figure 36). Injections of dextran amines in this part of nidopallium yielded Golgi-like retrograde label of neurons in dorsal thalamus, probably because of the dense terminals as shown by lentiviral injections. It is likely that this region of nidopallium is the same area considered part of the "shell" pathways (Bottjer and Altenau 2010). As reviewed in the Introduction (section I.2.D.2), some behavioral studies suggest the shell pathway is involved with song, perhaps relating to beak movements (Mandelblat-Cerf, Las et al. 2014). Below I discuss the functional implications of this and compare this possible pathway to what I reviewed about projections of motor thalamus and intralaminar thalamus in mammals.

IV.2 Functional implications and future studies

The demonstration that there are thalamostriatal projections in the song system raises the question of what function these projections have. There already exists an extensive literature on proposed functions of the thalamostriatal system in mammals, reviewed in the Introduction (section I.2.A.4), and this literature includes specific predictions for functions the thalamostriatal system might play in the song system. In the following sections I propose follow-up experiments, providing a rationale based on previous literature. I then move on to do the same for the results suggesting a pathway from cerebellum to Area X through dorsal thalamus. Although less is known about the disynaptic pathway from the cerebellar nuclei to the striatum in mammal, I base my predictions on previous literature to the extent possible. In closing, I predict likely outcomes of these follow-up studies based on previous literature and my own results, and discuss the functional implications.

IV.2.A Thalamostriatal projections

IV.2.A.1 Microcircuitry of Area X, including thalamostriatal projections: ultrastructural studies

The addition of a projection from dorsal thalamus to Area X raises the question of where and how this input is integrated in relation to other inputs to Area X. My results with lentiviral vectors provide strong evidence for a thalamostriatal projection within the song system, but a demonstration that these synapses exist with electron microscopy (EM) would provide incontrovertible evidence, and also make it possible to answer questions about how thalamostriatal inputs are integrated with other inputs to Area X. Below I discuss determining cell types in Area X targeted by thalamostriatal projections, but for the moment I assume that, as in mammals, the

major target of thalamostriatal projections is MSNs. If that is the case, it raises the question of where on the dendritic tree thalamostriatal terminals are found. Recall that in mammals, thalamostriatal terminals from the CM/Pf preferentially form on the dendritic shaft, while terminals from CL (and presumably other regions outside CM/Pf) preferentially form on spines, as do corticostriatal terminals. Note that a recent study did find that single Pf neurons could in some cases form synapses on just the dendritic shaft, just the spines, or in both locations (Lacey, Bolam et al. 2007). Terminals on spines are thought to be biochemically isolated from the rest of the cell so that their strength can be changed independently (Diamond, Gray et al. 1969, Nimchinsky, Sabatini et al. 2002). It is unclear what physiological role is played by axo-shaft synapses, in the thalamostriatal system (Smith and Bolam 1990, Parker, Lalive et al. 2016) and in other brain areas such as cortex (Araya, Eisenthal et al. 2006, Zheng and Schwabe 2015). Knowing the location of thalamostriatal terminals on Area X MSNs would answer questions about their similarity to such terminals in mammals, and put constraints on models that have proposed functions for thalamostriatal synapses based on their location on the dendrite (Fee 2012). To determine where thalamostriatal terminals form on Area X MSN dendritic trees, I would combine viral injections in DLM and DT_{CbN} with EM. By making injections of AAV-GFP in dorsal thalamus, I could obtain strictly anterograde label of thalamostriatal projections that I could then prepare for EM processing, combining a GFP-directed antibody with DAB staining to yield an electron dense product. I would determine whether synapses form with dendritic shafts or spines at the EM level by measuring diameters of post-synaptic processes adjacent to stained terminals. This criterion is what is currently used in studies now, although if necessary I could as in the original studies (Kemp and Powell 1971) carry out Golgi staining and verify at the light

microscopy level that I was examining MSNs adjacent to thalamostriatal terminals before processing for EM and then measuring post-synaptic terminals of the Golgi-stained MSNs. Using similar techniques I would also determine where LMAN and HVC terminals are on MSNs. To my knowledge, there are currently no studies that test where LMAN or HVC synapses are found on MSNs, although this would be relatively simple to determine with standard tracer injections and EM. Lastly I would ask where all three types of terminals are in relation to dopaminergic terminals by staining the dopaminergic fibers with a tyrosine hydroxylase antibody. The previous literature in mammals also suggests that thalamostriatal terminals on spines are located close to dopaminergic fibers, while the CM/Pf terminals on the dendritic shaft are not (Smith, Bennett et al. 1994, Moss and Bolam 2008). Taken together, these studies would demonstrate anatomically whether Area X MSNs integrate thalamostriatal inputs similarly to corticostriatal inputs from LMAN and HVC, and which of those inputs is subject to modulation by dopaminergic input.

IV.2.A.2 Topography and morphology of thalamostriatal projections: single-cell reconstruction studies

My results showing there are thalamostriatal projections raise questions that can best be answered by single-cell tracing studies. For example, do single neurons in DLM project to both Area X and LMAN, i.e. striatum and cortex? As reviewed in the Introduction (section I.2.A.4), the literature currently identifies two classes of thalamostriatal neuron in mammals, one type in the CM/Pf and another type found in regions of thalamus outside of CM/Pf. Several characteristics differentiate these two classes, suggesting each class has a different functional role. Much of the evidence for characteristics that distinguish the two classes comes from single-neuron tracing studies. To answer questions about the morphology of dorsal thalamic neurons, I would carry out single-cell

reconstruction studies. Many such studies have been performed by juxtacellular iontophoresis of biocytin of the neuron followed by DAB staining and then reconstructing the neuron using light microscopy (Pinault 1996). This method may not work in the song system because each DLM neuron is enveloped by one giant calyx-like terminals from an Area X projection neuron; it is possible the biocytin would retrogradely label the Area X pallidal neurons, and in fact the axons of individual Area X neurons have been traced this way previously with dextrans (Gale, Person et al. 2008). More recent studies have used viral vectors that are highly diluted to increase the chance that only one cell is infected, so that it can then be reconstructed (Kuramoto, Furuta et al. 2009). This would provide an alternative approach if biocytin does not work. Upon finding an approach that works, there are several morphological questions I would answer. I would first determine whether the dendritic trees of thalamostriatal neurons have a highly-branching "bushy" morphology typical of thalamic relay neurons and thalamostriatal neurons outside CM/Pf, or whether the dendritic trees instead have a "reticular"-like morphology with few branches, as do CM/Pf thalamostriatal neurons. Such a result would provide an important clue about how these thalamic neurons integrate inputs. Especially given that DLM neurons are already known to generate LTS bursts typical of thalamic relay neurons (Person and Perkel 2005, Leblois, Bodor et al. 2009), it would be good to know whether neurons that form synapses in striatum generate such bursts. If they also have "bushy" dendrites, it would support the idea that they belong to a distinct class of thalamic neuron. It will also be important to quantify the morphology of thalamic axon collaterals in striatum. Recall that in mammals the CM/Pf class sends out several collaterals within striatum that ramify locally to form dense clusters of terminals (Deschenes, Bourassa et al. 1996), while neurons in CL (and, it is thought, other parts of thalamus outside CM/Pf) can have many

long branches with varicosities that form en passant terminals throughout large patches of the striatum (Deschenes, Bourassa et al. 1996), instead of the heavily branched terminals of CM/Pf neurons that appear to focally target regions. These are qualitative differences, but they can be quantified in terms of axon length and number of branches. Such quantification will be important if thalamostriatal neurons in the song system have both long axons with varicosities and focal branches of axon terminals as has been reported for motor thalamus in primates (McFarland and Haber 2001). Quantifying individual neurons will make it possible to ask whether there are subtypes. Lastly, I would describe the extent of terminals in cortex relative to striatum—i.e. whether collaterals in striatum are sparse while axon terminals form dense branches in LMAN or nidopallium just outside LMAN. In mammals, the CM/Pf neurons that form dense local foci of terminals in striatum send only sparse projections to cortex, while CL neurons that have long varicose axons without heavily branched regions in striatum are the ones that send dense projections to cortex (Deschenes, Bourassa et al. 1996, Deschenes, Bourassa et al. 1996). Branching in cortex should also be quantified to compare with striatum. The differences in morphology in mammals (Deschenes, Bourassa et al. 1996, Deschenes, Bourassa et al. 1996) suggest that the CM/Pf class of thalamostriatal neuron, with its focally dense terminals in striatum and sparse projections to cortex, may play a more direct role in regulating the basal ganglia compared to other parts of thalamus that synapse with neurons in striatum *en passant* but have dense axon terminals in cortex. These differences raise the question of how heavily neurons in songbird dorsal thalamus project to striatum versus cortex. There is also the possibility that individual neurons may project only to Area X or only to LMAN, similar to how a subset of CM/Pf neurons in primates projects to only striatum or only cortex (Parent and Parent 2005), and this

would also be answered by single-axon tracing studies. Another question that would be addressed is whether thalamostriatal neurons send collaterals to other regions such as ventral pallidum (a target of Area X output and a source of VTA input) and the thalamic reticular nucleus—CM/Pf send collaterals to the thalamic reticular nucleus as well as the globus pallidus, and if there are similar collaterals in songbird thalamostriatal neurons, it would suggest these projections modulate activity in multiple areas instead of relaying some specific information to Area X. One last question to answer relates to the EM studies described above. Lacey et al. (2007) found that individual thalamostriatal neurons in CM/Pf differ in the extent to which they target dendritic shafts or spines of MSNs, by combining single-cell tracing with EM. If individual neurons of songbird dorsal thalamus vary in what regions of the dendrite they target, then it may not make sense to think of thalamostriatal neurons as a group that preferentially targets dendritic shafts as a whole and by doing so regulates MSN activity differently than corticostriatal inputs that target MSN spines. An alternative explanation would be there are subtypes of thalamostriatal neuron that differ in how they influence Area X MSN activity (depending on whether they preferentially target spines or dendritic shafts). The various types of findings just described—morphological and ultrastructural—based on single-cell reconstruction would, like the studies in the previous section, place constraints on possible models of the function of Area X, such as the model of how Area X MSNs integrate input (Fee and Goldberg 2011, Fee 2012).

IV.2.A.3 Cell types in Area X targeted by thalamostriatal projections: immunohistochemical studies

Another question raised by the result showing a thalamostriatal projection to Area X is: what cell types are targeted by that projection? Obviously, hypotheses about the function of thalamostriatal

projections in the song system should be informed by the cell types in Area X that those projections target. It is known in mammals that projections to striatum from the CM/Pf target several interneurons in addition to MSNs, while projections from other parts of thalamus have only been shown to target MSNs, as reviewed in the Introduction (section I.2.A.4). Previous studies in mammals also show it will be necessary to combine immunohistochemistry with tracer injections and EM to clearly show which Area X cell types are targets of thalamostriatal projections (Lapper and Bolam 1992). One way it might be possible to identify MSNs would be to use an antibody against DARPP32, a known marker of MSNs in Area X (Rochefort, He et al. 2007, Garcia-Calero and Scharff 2013) although this antibody is produced by a research lab and not available commercially. I consider it likely that thalamostriatal neurons target MSNs, simply because they MSNs are the most common cell type in Area X, and because my other findings combined with previous work suggest that thalamostriatal neurons in DLM and DT_{CbN} are more similar to CL neurons that mainly target MSNs, than CM/Pf neurons. Hence I would first test for this connection, but if there were a number of thalamostriatal synapses not contacting MSNs, I would then move on to other cell types. Most notably, work in mammals has shown that CM/Pf thalamostriatal projections target acetylcholinergic interneurons. Such neurons are found in Area X (Carrillo and Doupe 2004, Reiner, Laverghetta et al. 2004) and can be labeled with currently available antibodies as I have shown. A result showing thalamostriatal projections target acetylcholinergic interneurons in Area X would suggest the functions of these projections might be more related to modulatory systems than relaying specific motor or sensory information. It is known that somatostatin and parvalbumin-containing interneurons are also targeted by CM projections in monkeys (Sidibe and Smith 1999), but calbindin containing interneurons are not. I have

successfully stained Area X with parvalbumin as reported here. Again based on current results that suggest thalamostriatal projections in songbirds are more like CL-type thalamostriatal neurons in mammals, this would not be the first hypothesis I would test, but it would be on the list if thalamostriatal synapses were not all targeting MSNs. No function has been ascribed to this targeting of inhibitory interneurons of striatum in mammals. Lastly, it will be important to test whether thalamostriatal projections synapse with the pallidal-like output neurons of Area X. To my knowledge, these neurons have not been studied at the EM level, but their dendrites have a distinctive beads-on-a-string morphology which presumably is also distinctive when seen in EM material. Even if that is not the case, the output neurons are easy to retrogradely label by injecting dextrans in DLM (Reiner, Laverghetta et al. 2004). Note that if combined with viral methods to label thalamostriatal projections, this would then lead to double-label of the thalamic neurons. The DLM-projecting Area X cells can also be labeled with antibodies against LANT6 (Reiner, Laverghetta et al. 2004), but this is another antibody produced by a research lab that may not be available. An alternative is antibodies against GAD, although this will also label all inhibitory neurons including MSNs (Luo and Perkel 1999, Luo and Perkel 1999). Regardless of how the Area X neurons are labeled, if a reciprocal connection were found between these Area X neurons and the DLM neurons that they target, it would be perhaps the most surprising result of any suggested here. It would be particularly interesting if neurons in DLM make en passant with pallidal neurons and MSNs in addition to the axon terminals they form in LMAN, because it would raise the question of what function reciprocal and/or recurrent connections play within the AFP. I discuss possible functions of these reciprocal connections below in section IV.2.A.6.

IV.2.A.4 Subcortical inputs to dorsal thalamus: retrograde tracer and immunohistochemical studies

If songbird dorsal thalamus really is homologous to the intralaminar nuclei as previously hypothesized (Medina and Reiner 1994, Veenman, Medina et al. 1997), then like the intralaminar nuclei it should be receive ascending input from the brainstem and midbrain. Recall that in mammals it is thought that thalamostriatal projections are thought to relay input from the brainstem and superior colliculus that communicate motor efference copies (Winn, Wilson et al. 2010, Fee 2012, Smith, Galvan et al. 2014) and context/state/ "surprise" (Minamimoto, Hori et al. 2009, Bradfield, Hart et al. 2013). It would be particularly interesting if dorsal thalamus were the target of such ascending inputs because the thalamostriatal projections I have provided evidence before could similarly relay efference copies or state information to Area X. Thus, if songbird dorsal thalamus is homologous to the intralaminar nuclei, it should receive input from the brainstem reticular formation, including the acetycholinergic and serotonergic cell, as do the intralaminar nuclei (Hallanger, Levey et al. 1987, Pare, Smith et al. 1988, Steriade, Pare et al. 1988, Smith, Galvan et al. 2014). Surprisingly, I find no study that has tested with retrograde tracers whether thalamic song system nucleus DLM receives subcortical inputs, acetylcholinergic or otherwise. Injections of dextran amines in DT_{CbN} described in this dissertation did not yield obvious retrograde label of subcortical structures (besides the cerebellar nuclei) but this could be due to the nature of the tracer used. Studies in pigeons have involved injections of other dorsal thalamic nuclei but I also find no report in these studies of inputs similar to the Ascending Reticular Activation System described in mammals (Van der Werf, Witter et al. 2002). As reviewed in the Introduction (section I.2.B), some inputs from optic tectum to dorsal thalamus in pigeons have

been described (Korzeniewska and Güntürkün 1990, Wylie, Glover et al. 1998), and these inputs may reach DT_{CbN}. Retrograde tracer injections in DLM and DT_{CbN} would also permit testing whether either region of dorsal thalamus receives subcortical input that could provide an efference copy (Winn, Wilson et al. 2010, Fee 2012, Smith, Galvan et al. 2014). In the case of the mammalian oculomotor system, this efference copy is supposed to be relayed by the superior colliculus. But the superior colliculus is also proposed as the region that activates the intralaminar nucleus in response to "surprising" stimuli (Minamimoto, Hori et al. 2009), as reviewed in the Introduction (section I.2.A.4), so a positive result showing that DLM or DT_{CbN} receives input from superior colliculus would not immediately have a clear meaning for function. But any results about subcortical inputs would be a helpful contribution to the literature. It will be important if such studies are carried out to also make injections in the subcortical nuclei to demonstrate anterograde label in DLM and DT_{CbN}, i.e. to show that retrograde label did not result due to uptake by fibers of passage. This can especially be a confound for brainstem modulatory cell groups that send projections throughout the brain. Even results showing little or no projections from subcortical regions would be useful because they would imply that dorsal thalamus might be more like motor thalamus in mammals than the intralaminar nuclei, although this would admittedly be an argument from absence of evidence.

IV.2.A.5 Efferents of dorsal thalamic subregions: studies with iontophoretic injections of AAV

My results suggest that neurons in DLM and immediately-adjacent subregions of DT_{CbN} both form axon terminals in Area X (Figure 20). However, the lentiviral vector required a large volume of solution to be injected and some spillover into neighboring subregions is unavoidable. I could not

rule out the possibility that DLM projects to Area X while DT_{CbN} projects only to nidopallium. This question can be more cleanly addressed by using an AAV vector. My initial experiments suggest that AAV vectors do work in songbirds. Like lentiviral vectors, AAV vectors yield strictly anterograde label (except when engineered to travel in the retrograde direction) but unlike lentiviral vectors, AAV can injected iontophoretically (Harris, Wook Oh et al. 2012); such injections are smaller and can be cleanly confined to DLM or DT_{CbN}. Another possible confound arising from use of the lentiviral vector is that ectopic (over)expression of synaptophysin induces formation of synapses that are not naturally occurring, although this has not been reported previously (Grinevich, Brecht et al. 2005, Bauer, Coleman et al. 2008, Roberts, Klein et al. 2008). This possibility would be less likely if varicosities and terminal-like processes are still observed in Area X after injecting an AAV vector into DLM or DT_{CbN} that simply expresses GFP or another fluorescent protein without overexpressing synaptophysin. If results did show that DLM projects to both Area X and LMAN, while DT_{CbN} projects only to nidopallium, this would provide more evidence that dorsal thalamus more closely resembles mammalian motor thalamus, where presently the literature suggests that the basal ganglia are reciprocally connected but cerebellar output to motor thalamus is relayed strictly to cortex (Kuramoto, Furuta et al. 2009), as reviewed in the Introduction, and unlike the case for the intralaminar nuclei where cerebellar output passes through CL to dorsolateral striatum (Deschenes, Bourassa et al. 1996, Ichinohe, Mori et al. 2000).

IV.2.A.6 Physiology of thalamostriatal projections

It will be important to determine physiologically what effect projections from dorsal thalamus have on their targets in Area X. Before I describe experiments to investigate those effects, I point out

that the single-cell tracing experiments outlined above also provide the opportunity to record activity of thalamostriatal neurons, at least under anesthesia. Single-cell tracing studies in rats found that CL neurons with relay-like bushy dendritic morphology often produced LTS bursts as identified in extracellular recordings, while CM/Pf neurons produced such bursts rarely (Lacey, Bolam et al. 2007). Since there has been little evidence for these bursts playing a role in behavior in mammals (Bosch-Bouju, Hyland et al. 2013), the functional implications would be unclear, but such a finding would be evidence that thalamostriatal neurons in DLM or DT_{CbN} are at least similar to one class of such neuron in mammalian thalamus. Recall that there are questions about whether Area X output controls neurons in DLM by eliciting rebound spiking with large inhibitory post-synaptic potentials (Person and Perkel 2005, Leblois, Bodor et al. 2009), as reviewed in the Introduction (section I.2.A.3.d). In songbirds it also seems to be the case that neuronal activity in DLM during song consists of high-frequency spikes, not LTS bursts (Goldberg, Farries et al. 2012), but it would still be informative to know whether DLM and DT_{CbN} thalamostriatal neurons belong to the class capable of LTS spikes. More important is the question of how thalamostriatal input effects striatal activity. Although slice physiology studies are challenging in birds, this would be the best method to compare several characteristics shown to be important in mammals (Ellender, Harwood et al. 2013). Here again I assume for now the main target of thalamostriatal projections to Area X is MSNs, and that thalamostriatal inputs are excitatory and glutamatergic as they are in mammals. In slice I would compare the amplitude of thalamostriatal EPSCs to those from cortex to see whether either appears to be a significant driver of MSN activity. I would also measure paired-pulse ratio of thalamostriatal inputs to determine whether they are likely to facilitate MSN firing. Then I would determine whether the thalamostriatal EPSCs are mainly

AMPA or NMDA receptor-mediated by applying AP5 to measure only the AMPA current and then subtracting this from total current to obtain the NMDA current. Lastly I would look at long-term plasticity of thalamostriatal synapses by recording from them after pairing with stimulation of cortical synapses. Based on slice studies in mammals (Ellender, Harwood et al. 2013) and my own anatomical results suggest thalamostriatal projections in songbirds have morphology more like the non-CM/Pf thalamostriatal neurons in mammals, I predict that thalamostriatal synapses will be more like CL inputs to MSNs in mammals: large-amplitude and facilitating EPSCs that are AMPA-receptor mediated and do not display long-term plasticity. These findings would be consistent with a role for thalamostriatal projections in driving activity but not in directly controlling plastic changes involved with learning that takes place in the AFP.

Ultimately, the question will be to what extent these results hold up in a singing bird. There are two related questions I would test: whether thalamostriatal projections normally influence activity of MSNs during singing, and if so, how does that contribute to the function of the AFP. Answering both of these questions is complicated by the likely case that neurons in DLM (or DT_{CbN}) send collaterals to Area X but also terminate in LMAN, which itself sends collaterals to MSNs in Area X. If a single MSN can be excited by thalamostriatal synapses and by corticostriatal neurons from LMAN neurons that are also excited by the same neurons in thalamus, then a method is needed to excite and inhibit thalamostriatal synapses without having an effect on thalamocortical synapses from the same neuron. It might be possible to inhibit thalamostriatal synapses selectively with light-activated channels archaerhodopsin (Arch) (El-Gaby, Zhang et al. 2016) or halorhodopsin, although this has not yet been tested in songbirds, and there are reports of issues with long-term

activation of archaerhodopsin in mammals (Mahn, Prigge et al. 2016). Assuming that it works, I would first combine optical activation of Arch with extracellular recordings in singing birds to test whether inhibiting thalamostriatal inputs affects firing of MSNs in Area X during song.

If I could demonstrate that MSN activity is influenced by thalamostriatal input, I would then test a specific hypothesis about the function of this projection. This hypothesis relates to one function of the AFP, to contribute variability to song (Kao, Doupe et al. 2005, Kao and Brainard 2006). This variability is thought to be required for the bird to explore motor space as in reinforcement learning paradigms. It is not known how or where in the AFP this variability is generated, although it is known that the output from cortical nucleus LMAN in the AFP to motor cortex analog RA is required for this component of variability in song (Kao, Doupe et al. 2005, Kao and Brainard 2006). Crucially, for the brain to add variability to motor commands, the noise in neural activity must be correlated across a population of neurons. Otherwise target areas that receive output from that population would integrate uncorrelated noise that in effect would cancel itself out. A recent modeling study found that, in order for an artificial neural network to generate variability that is correlated between groups of neurons, it must have both topographically organized projections between layers and recurrent projections within layers (Darshan, Wood et al. 2017) (recurrence in this context mean connections within a layer, analogous to local collaterals in a brain region). It is known that the AFP is topographically organized (Johnson, Sablan et al. 1995, Luo, Ding et al. 2001) including the collaterals that LMAN sends back to Area X (Nixdorf-Bergweiler, Lips et al. 1995, Vates and Nottebohm 1995). I hypothesize that the *en passant* synapses that single thalamostriatal axon collaterals form with multiple neurons in Area X contribute to correlations in

variability across neurons. Testing this would require specifically inhibiting thalamostriatal synapses, perhaps with an optogenetic method, while recording from multiple neurons in Area X during song. My prediction would be that the covariance across neurons would decrease when thalamostriatal synapses are silenced.

IV.2.A.7 Determine nature of information relayed to DLM and DTCbN by subcortical inputs

I stated above that it is not known if there are subcortical inputs to DLM or DT_{CbN} (besides the projection from the cerebellar nuclei) and that if, as has been proposed, dorsal thalamus is homologous to the intralaminar nuclei, it should receive inputs from the superior colliculus and brainstem neuromodulatory cell groups. There is evidence that both areas activate the intralaminar nuclei in response to unexpected stimuli (Minamimoto, Hori et al. 2009, Bradfield, Hart et al. 2013). This activation is thought to "rebias" basal ganglia calculations about reward in response to surprising stimuli (Minamimoto, Hori et al. 2009), or help learn new response-outcome associations (Bradfield, Hart et al. 2013). It has also been proposed that the superior colliculus might also relay motor efference copies (Fee 2012). There is some evidence for motor-related activity in regions of superior colliculus (McHaffie, Stanford et al. 2005) known to project to the intralaminar nuclei (Krout, Loewy et al. 2001). One of those intralaminar nuclei, MD, displays activity during saccades consistent with the idea that it relays corollary discharge (Sommer and Wurtz 2002), i.e. an efference copy of motor commands, and MD could relay that activity to MSNs via its thalamostriatal projections. Assuming that I found positive evidence for subcortical inputs with neuroanatomical studies, a logical next step would be to determine the nature of the information conveyed to dorsal thalamus by such inputs. A first step to determine this would be to

record from antidromically-identifed neurons in these subcortical areas that projecting to DLM or DT_{CbN} during an aversive auditory feedback paradigm like that described in the Introduction (section I.2.A.3.e). If neurons fire in response to white noise blasts, this would be evidence they convey context information, but if they do not change firing in response to white noise blasts but are correlated with acoustic parameters of song, this would support the idea that they convey efference copies of motor output.

IV.2.B Cerebellothalamic projections

Here I propose several follow-up studies to further investigate the function of cerebellothalamic projections and the cerebellum in song. It is tempting to think the cerebellum would be involved with learning and producing birdsong, because of the overwhelming evidence that demonstrates the cerebellum is required for co-ordinated execution of motor skills. However, as reviewed in the Introduction (section I.2.A.5), it remains an open question how the cerebellum contributes to motor skills, and many functions have been proposed. To name but one, there are many studies of the cerebellum's role in timing (Perrett, Ruiz et al. 1993, Mauk, Medina et al. 2000, Medina, Garcia et al. 2000). Strikingly, control of timing in the song system does not depend on the thalamocortical-basal ganglia pathway (Ali, Otchy et al. 2013) discussed at length above (section I.2.A.3. In spite of the obvious question of whether the cerebellum is involved with learning and producing birdsong, there are few published studies of the cerebellum's role in motor behavior in songbirds (Spence, Zhen et al. 2009, Hall, Street et al. 2013), and none of them deal directly with song. The studies I propose here are arranged (roughly) in order from those that immediately build on the current results to those that are more exploratory. Some of the hypotheses are even

contradictory. The main intent here is to summarize possibilities for future research, with pointers to relevant literature.

IV.2.B.1 Is the projection from CbN to thalamus glutamatergic? Can immunostain for glutamatergic and GABAergic inputs to dorsal thalamus serve as a proxy for pallidal and cerebellar inputs? A combined tracer and immunohistochemical study.

Previous studies in mammals have shown that the territories of ventral motor thalamus can be divided into inhibitory pallidal-recipient zone and an excitatory cerebellar-recipient zone. In mammals, GAD67 antibodies can be used to stain the pallidal inputs and VGLUT2 antibodies can be used to stain the cerebellar inputs. Studies that combined tracers with immunohistochemistry confirmed that cerebellar axon terminals are immunoreactive for VGLUT2, a receptor found at many types of subcortical excitatory axon terminals, and that pallidal terminals are immunoreactive for GAD67, one enzyme that converts glutamate to GABA. Lesioning either the cerebellar nuclei or globus pallidus with ibotenic acid resulted in loss of the corresponding immunosignal at the terminals in motor thalamus (Kuramoto, Furuta et al. 2009, Kuramoto, Fujiyama et al. 2011). A similar approach was used with GAD65 antibodies and neurotoxic lesions to show that output from Area X to DLM was inhibitory. Unfortunately there are currently no commercially available antibodies for VGLUT2 in birds. One group has cloned an antibody against VGLUT2 from pigeons, and this antibody has been used to study the distribution of VGLUT2 in a songbird, the zebra finch, but this study did not examine the nature of specific projection types (Atoji 2011, Karim, Saito et al. 2014) such as the thalamostriatal projection. Note that in the mammalian brain VGLUT2 is also found at thalamostriatal terminals, whereas

VGLUT1 is found at corticostriatal terminals, so antibodies for both can be used to label those terminals and analyze them at an ultrastructural level without needing to perform tracer injections (Raju, Shah et al. 2006). Although it is highly likely that the cerebellar projection to dorsal thalamus is glutamatergic, it would be helpful for later studies to demonstrate this by similarly combining some immunohistochemical stain with tracer injections in CbN.

IV.2.B.2 Disynaptic pathways from CbN to the forebrain through thalamus: a study with transsynaptic tracers

The studies in this dissertation provide some evidence that subregions of DT_{CbN} projects to Area X (Figure 20), medial striatum outside Area X (Figure 21), and nidopallium (Figure 36). The results do not demonstrate whether there is a disynaptic route through DT_{CbN} from CbN to Area X, analogous to the route through intralaminar nucleus CL from CbL to dorsolateral striatum in mammals (Ichinohe, Mori et al. 2000, Hoshi, Tremblay et al. 2005). As explained above in the Discussion (section IV.1.B), a disynaptic pathway from CbN to Area X through DT_{CbN} in particular is hard to demonstrate (see also Appendix V.4.A, Figure 33), because of passing fiber confounds and other technical issues such as lack of retrograde label throughout dendrites from injections in Area X, and the presence of a direct projection from Area X to DLM. An alternative approach to paired tracer injections would be to test for disynaptic pathways from CbN to the forebrain through thalamus using transsynaptic tracers.

The standard transsynaptic tracer, rabies virus, does not work in birds (personal communication, Roberts and Strick). There are two alternatives I am aware of: a “purely” lentiviral approach, and a viral approach that induces expression of wheat germ agglutinin. Unfortunately there is previous

evidence both are problematic. The lentiviral approach employs vesicular stomatitis virus and its glycoprotein (VSV-G) and has been shown to work in many vertebrates including chickens (Mundell, Beier et al. 2015). Noticeably absent from that methods paper was a demonstration that the virus works in songbirds. This may be because finches in particular (the most commonly studied songbird species) lack the LDL receptor that has been shown to be the receptor that takes up the VSV-G virus (Finkelshtein, Werman et al. 2013)—finches do have a truncated orphan receptor with high sequence similarity to LDL and this may explain why lentiviral approaches can work but often with lower efficiency than what is seen in mammals (Mello, personal communication). The approach that induces expression of wheat germ agglutinin has specifically been shown to *not* work in the pathway from the cerebellar nuclei to striatum via thalamus in mice (Braz, Rico et al. 2002), and so it would likely not work in birds. So these methods are probably not at the moment suitable, but I want to make note here of the alternate approach and what is known about them.

IV.2.B.3 Specificity of projections of the cerebellar nuclei: tracer studies

Does the cerebellum convey specific information to parts of dorsal thalamus that project to Area X? My neuroanatomical results show that CbN projects to a broad mediolateral swath of dorsal thalamus, including: a region adjacent to DLM that seems to also target Area X (Figure 20) and a more medial and posterior region that targets medial striatum (Figure 21). It is unknown whether single neurons in CbN project to all of these subregions of thalamus. Similarly the results showed that CbL in particular targets many other areas besides dorsal thalamus, including the red nucleus, SpM, and PPC. It is again unknown whether single neurons in CbN project to all of these regions,

although as reviewed in the Introduction (section I.2.A.5.a.1), single-axon tracing studies suggest there are neurons in mammalian CbN that send collaterals to the red nucleus *en route* to thalamus (Shinoda, Futami et al. 1988).

Future anatomical work should determine whether neurons in CbN project to more than one subregion of DT_{CbN}, and also determine whether they project to other regions outside of thalamus such as the red nucleus. One approach to determining whether neurons in CbL project to both dorsal thalamus and the red nucleus is to make injections of retrograde tracer in both target regions. If there are few or no double-labeled neurons in CbN, this would suggest there are two separate pathways from the cerebellum to thalamus and the red nucleus. A next obvious question would be whether neurons in these separate pathways receive segregated inputs from cerebellar cortex. The question of whether single neurons in CbN target multiple regions of dorsal thalamus could also be addressed by retrograde tracer injections with multiple fluorophores in different regions of dorsal thalamus. Interestingly similar studies in primates suggest this may not be the case for the projection of the cerebellar nuclei to motor thalamus (Craig 2008). A complementary approach would be single-axon tracing studies like those described previously (Shinoda, Futami et al. 1988). Such studies would rule out the possibility that double-label from the retrograde tracer studies arose due to fibers of passage, and also show whether single CbN neurons send collaterals to multiple areas in thalamus or the midbrain.

IV.2.B.4 Does the cerebellum interact with VTA?

VTA in songbirds provides dopaminergic input to Area X (Bottjer, Halsema et al. 1989, Person, Gale et al. 2008), and is thought to relay performance errors during song (Gadagkar, Puzerey et al. 2016, Hoffmann, Saravanan et al. 2016). Previous anatomical studies report that the medial cerebellar nuclei projects to VTA in rats (Snider and Maiti 1976) and unpublished work from the Khodakhah group found that this projection may be involved with social behaviors. VTA in pigeons is also reported to be a target of the cerebellar nuclei (Arends and Zeigler 1991). If the cerebellar nuclei were to project to VTA in songbirds, this would obviously provide a monosynaptic path through which cerebellar output could modulate the song system, and perhaps provide VTA with a source of signals about performance error.

To establish a connection between VTA and the cerebellar nuclei in songbirds anatomically, I would carry out two experiments. First I would inject retrograde tracer in VTA and based on my hypothesis expect to see retrograde label in the cerebellar nuclei. Then to confirm the projection exists, I would make iontophoretic injections of AAV-GFP vector into the regions of the cerebellar nuclei where I saw retrograde label after injecting in VTA. In the same birds, I would inject retrograde tracer in Area X to label X-projectors in VTA. If output from CbN can reach Area X through VTA, I should see axon terminals from the cerebellar nuclei closely apposed to the dendritic tree of neurons in VTA that project to X. Obviously this requires that the retrograde tracer strongly label dendrites. Injections of dextran amines provide Golgi-like retrograde fill but when injected in Area X do not label VTA (Person, Gale et al. 2008, Nicholson, unpublished work). Cholera toxin B does yield retrograde label of VTA when injected in Area X, but the label is

granular and mainly confined to the soma (Person, Gale et al. 2008, Nicholson, unpublished work). One approach would be to combine cholera toxin B injections with immunohistochemistry for tyrosine hydroxylase (TH), because TH stain robustly labels the entire cell body of dopaminergic neurons in VTA that project to Area X, including dendrites. Another approach would be to use a retrograde AAV-GFP viral vector, which I have shown previously can label the VTA projection to Area X and results in expression of GFP throughout the dendritic tree. Predictions on the function of this pathway would depend on the regions of CbN that project to VTA. In mammals, both the fastigial and interpositus nucleus have been reported to project to VTA. One possibility is that the medial and interpositus regions could relay information about body movements to VTA during song (see related proposed experiments in IV.2.B.4).

Assuming a positive result from the neuroanatomy, the next step would be to investigate the function of this pathway physiologically. I would first show functional connectivity. One approach would be to inject AAV-ChR2 in regions of CbN that project to VTA, then record from X-projecting neurons in VTA while stimulating CbN terminals in VTA optically. If I found that cerebellar output can influence activity in VTA during song, I would then test for hypothesized functions of this interaction between the two regions. Based on what is known about cerebellar function, I would predict that CbN provides an estimate of errors to the VTA (but not an error signal. Error signals that induce plasticity at synapses within the cerellum are generally thought to arise from climbing fibers of the inferior olive that elicit complex spikes (CSs) from Purkinje cells, although recent work suggests CSs may actually be predictive and modulate ongoing Purkinje cell activity, see (Streng, Popa et al. 2017) and citations therein). Recent results using the white noise

paradigm described above (section I.2.A.3.e) suggest that the learning seen in this paradigm may have less to do with auditory feedback than with the aversive stimulus. The cerebellum could be one source that alerts the VTA to unexpected sensory feedback during song. To test for this I would record chronically from VTA during the white noise paradigm (section I.2.A.3.e), again using Arch to specifically inhibit terminals from CbN. Experiments have been done recording from Area X-projecting VTA during this paradigm (Gadagkar, Puzerey et al. 2016). I predict that, as shown previously, firing of X-projecting VTA neurons would be phasically depressed immediately following white noise blasts. I would further predict that inhibiting CbN terminals in VTA would prevent this response from occurring. If there were such a modulation of activity in VTA by CbN, these experiments would begin to answer long-standing questions about what kind of feedback the VTA uses to evaluate song.

IV.2.B.5 Disynaptic pathways from CbN to the forebrain through thalamus: physiological studies

If cerebellar output reaches Area X via a disynaptic pathway through DTCbN, or even if it reaches nidopallium through the same route and that part of nidopallium is involved with beak movement, the next questions become: how does cerebellar output affect activity in thalamus and the forebrain, and what functions do such effects have?

Based on the physiological studies of the cerebellothalamic pathways in primates reviewed in the Introduction (section I.2.A.5.a.2), one prediction that could be made is that firing activity in DT_{CbN} would convey an error-related signal to Area X or the nidopallium. Experiments that change auditory or somatosensory feedback to induce error would be necessary to test such a hypothesis.

Studies could also be carried out like those of Chen et al. (2014) that investigated how cerebellar output modifies the neural activity of MSNs via its projection to the CL in the intralaminar nuclei. Briefly: I would activate cerebellar projections to dorsal thalamus by electrical microstimulation or optogenetic excitation of CbN while recording from MSNs in Area X during song. Based on results from Chen et al. (2014) I would predict mixed effects on MSN firing activity (exciting some neurons but inhibiting others) but at a latency consistent with a disynaptic pathway. Because MSNs in Area X fire sparsely, and only during song, the metric to determine effects could not rely on a baseline firing rate (as Chen et al. used) but instead would have to be something that can be measured during bursts, e.g. two standard deviations outside the average instantaneous firing rate. To show that these effects indeed results from the pathway through dorsal thalamus, I would repeat the experiments but inactivate DT_{CbN} pharmacologically. Loss of effects on MSNs would demonstrate that they were in fact due to the pathway through dorsal thalamus.

IV.2.B.6 Does the cerebellum or red nucleus interact with phonatory or respiratory nuclei in the brainstem?

Tracer studies

As reviewed in the Introduction (section I.2.D), the literature does not currently provide any support for the idea that the cerebellum in songbirds interacts with brainstem motor nuclei that control phonatory or respiratory muscles. My results did show that CbL projects to red nucleus (Appendix V.2.C), and there is evidence from mammalian neuroanatomical studies that the red nucleus projects to jaw and respiratory motor nuclei (section I.2.A.5.a.3). One hypothesis to test would then be directly test whether any of these nuclei—trigeminal, hypoglossal, facial, and respiratory—receive afferents from the red nucleus. Of all hypotheses about cerebellar-brainstem

interactions this would probably be the easiest to test, by injecting anterograde tracer in the red nucleus and then injecting retrograde tracer in phonatory and respiratory muscles. The red nucleus is a large nucleus that would be relatively easy to target. Similarly, injecting retrograde tracers in muscles is an established technique (cf.,Wild 1993) that does not require targeting as precise as stereotaxic surgery. By comparison, injecting in tracer in the brainstem nuclei themselves would require some troubleshooting to find co-ordinates.

IV.2.B.7 Cerebellar involvement with beak movements: behavioral and physiological studies

I hypothesize based on my results and previous literature that the cerebellum is likely involved with beak movements in songbirds. This may seem surprising given the focus on disynaptic pathways from CbL to Area X in preceding sections of the discussion. Here I summarize the relevant results and explain what else would need to be done to show this involvement in terms of anatomy and physiology.

In terms of anatomy, I showed that the CbL in songbirds projects to dorsal thalamus, and that one subregion of DT_{CbN} projects to nidopallium. Double tracer injections in CbL and nidopallium showed that CbL axon terminals are closely apposed to dendrites of neurons in DT_{CbN} that project to nidopallium immediately outside LMAN (Appendix V.4.D, Figure 36). Viral vector injections in DT_{CbN} yielded strong label of axon terminals just dorsal to LMAN. It is very likely that some of this region is the area previously described as LMAN "shell" (Johnson, Sablan et al. 1995) that is supposed to receive input from DLM "shell", an area of dorsal thalamus described as ventral and medial of DLM. Note that some of the strongest label in dorsal thalamus after injections in

CbL was immediately ventral to DLM, in more medial regions of dorsal thalamus (Figure 16). It is not clear presently whether DT_{CbN} and DLM "shell" are the same regions, because in the original paper describing the shell pathways (Johnson, Sablan et al. 1995), brains were blocked for cutting caudal to thalamus and so any label from areas below thalamus was not described (e.g. retrograde label in CbN that would be expected to result from injections in DLM "shell" if it is the same region as DT_{CbN}). In follow up papers, it was shown that LMAN "shell" projected to an area outside song system motor cortex RA in arcopallium now known as Ai, and that one target of Ai is SpM, the pre-cerebellar nucleus (Bottjer, Brady et al. 2000). SpM sends major projections to lobule VI of cerebellum that in turn projects to CbL (Wild 1992). There is also behavioral (Mandelblat-Cerf, Las et al. 2014) and anatomical (Wild and Krützfeldt 2012) evidence that cortical region Ai is involved in control of beak movements (but see Bottjer and Altenau 2010), as I reviewed in section I.2.D. Briefly, anatomical results show that Ai neurons do not project directly to jaw muscle MNs but instead project to a pre-motor region in the brainstem in between nXIIts and RAm, RPcvm (ventromedial subregion of parvocellular (lateral) part of reticular formation), and this pre-motor region in turn projects onto the MNs innervating muscles controlling the jaws, syringes, and respiratory system (Wild and Krützfeldt 2012). This is very similar to the situation for vocal control in primates, where a brainstem pre-motor area co-ordinates the different motor nuclei (Jürgens 2002). Behavioral studies find that lesions of Ai may be involved with jaw movement, as the lesions affect both singing and eating in juveniles (Mandelblat-Cerf, Las et al. 2014).

To determine whether the cerebellum is involved with controlling beak movements would require several anatomical and physiological experiments. The first study to clarify my results would be to show whether areas of nidopallium that receive output from DT_{CbN} are in fact related to the shell pathway described by Johnson et al. (1995), I would first repeat the tract-tracing experiments with dual injections in CbN and nidopallium, but target the areas in nidopallium just dorsal to LMAN (not anterior) where I saw strong label after viral injections in DT_{CbN} (Figure 20, Figure 21). By making dextran amine injections in that area of nidopallium, I would not only retrogradely label areas in dorsal thalamus, but also anterogradely label projections of nidopallium to arcopallium. If this part of nidopallium does in fact project to Ai, then I should see anterograde label in that region. It will be important to section brains coronally to identify label in DT_{CbN} and Ai, to compare results with what was reported by Johnson et al. (1995) (where all brains were cut coronally). If in fact nidopallium dorsal to LMAN projects to Ai, this presents the possibility of a loop through cerebellum: CbL→Dt_{CbN}→nido→Ai→SpM→lobule VI→CbL (Figure 23). Additional anatomical

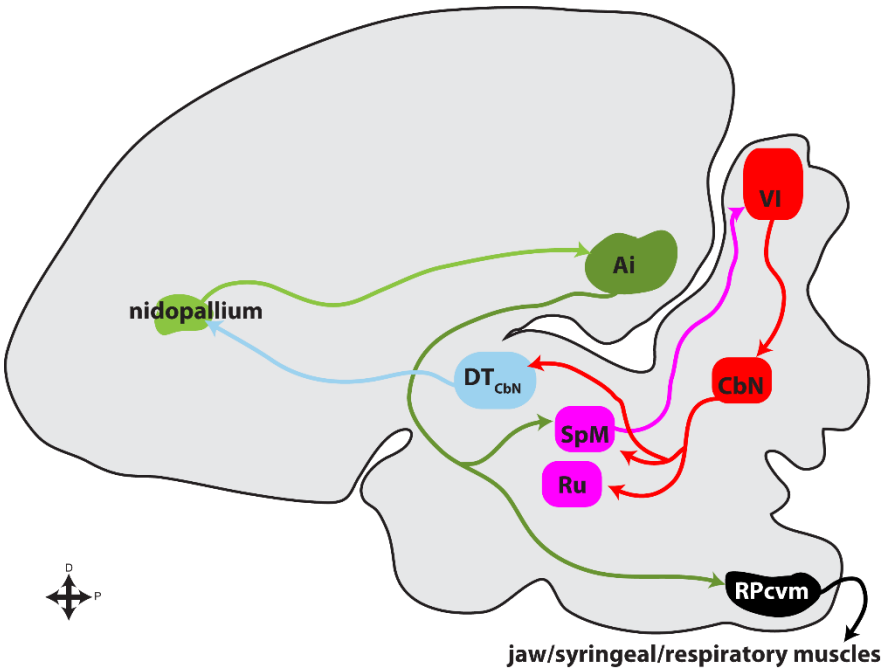

Figure 23. Hypothesized cerebro-cerebellar side loop involved in beak movements. Described in text.

experiments would be necessary to fully demonstrate that this loop occurs. The first would be to pair injections of dextran amines in areas of Ai where I saw retrograde label and in DT_{CbN}. If the pathway just outlined exists as hypothesized, this dual-tracer injection experiment should show in single animals that axon terminals from DT_{CbN} closely appose dendrites of nidopallial neurons that project to Ai. The same animals should have retrograde label in CbN, confirming the targeting of the correct part of dorsal thalamus and strengthening the argument that cerebellar output eventually reaches Ai. Also from those animals I will have anterograde label from the dextran amine injections in Ai. I would expect to see it in SpM, meaning that output from Ai can return to the cerebellum. Furthermore I would expect to see label in RPcvm, the brainstem region that is a known target of Ai and that co-ordinates activity of jaw, syringeal, and respiratory motorneurons (Wild and Krützfeldt 2012). A final question would be whether individual neurons in Ai project to both SpM and RPcvm, as this would provide one potential route for the cerebellum to receive efference copies from Ai. Providing evidence for this anatomically would require injecting retrograde tracer in RPcvm and SpM and looking for double-label of neurons in Ai.

If anatomical results do suggest that there is a cerebro-cerebellar "side loop" involved with beak control, a next obvious question would be whether this loop is involved with beak movements during song. Previous studies have quantified beak movement during song through video (Williams 2001, Podos, Southall et al. 2004) and through use of a magnetic transducer attached to the beak to measure gape (Goller, Mallinckrodt et al. 2004). Findings from these studies and others suggest that beak gape modulates the vocal tract, in effect allowing the bird to modify the amplitude of different frequencies across the spectrum during song (Nowicki 1987, Nelson,

Beckers et al. 2005, Riede, Suthers et al. 2006) , similar to the way that humans modulate the vocal tract by shaping the mouth and throat to emphasize different frequencies during speech. Direct evidence for beak gape modulating the vocal tract comes from a study that experimentally modified beak gape during song (Hoese, Podos et al. 2000). The authors found that amplitude in higher frequency ranges dropped when beak movements were restricted, and increased when beak gape was artificially increased. I hypothesize that the cerebellum is required to learn to control beak movements to filter spectral content during song. If this hypothesis is correct, it leads to the following two predictions: (1) lesioning CbL during development should specifically lead to poor acquisition of "pitchy" notes with only one or two harmonics in higher frequency ranges, with less effect on harmonic stacks with lower energy in higher frequencies, and (2) lesioning CbL in adult should not have gross effects on song. I specifically propose lesioning CbL instead of other regions in the possible cortical-cerebellar side loop because of previous reports that lesioning Ai resulted in gross impairment of singing and eating (Mandelblat-Cerf, Las et al. 2014), probably due to effects on beak movements. In contrast, cerebellar lesions in experimental animals do not typically result in total impairment, and the main effects are positive symptoms resulting from compensation. Bengalese finch song frequently contains a mix of "pitchy" whistle-like syllables and more "squawky" harmonic stack syllables, and therefore provides convenient features to test the hypothesis that the cerebellum is required to learn beak for accurately producing song.

IV.2.B.8 Is the cerebellum involved in sensorimotor learning or in social interaction?

In the Introduction, I emphasized that there are two important aspects of how juvenile songbirds learn their song: sensorimotor learning and social interaction (section I.1.A). I pointed to several

pieces of evidence that imply the cerebellum might be required in humans for normal development of communication and social skills (Wang, Kloth et al. 2014). Is it possible that the cerebellum is similarly required for the socially-mediated aspects of song learning in birds? Of course, because the cerebellum is well-known to be involved with motor control, it might not be clear whether any effect on song that arose from manipulations of cerebellum was due to impaired sensorimotor learning or alternatively due to impaired processing of social interaction. I outline an experiment that leverages the strengths of the songbird as a model system to differentiate between these two possibilities.

To determine whether the cerebellum is required for sensorimotor learning or socially-mediated imitation during development of song, I would compare how cerebellar lesions affect learning from two different song tutoring paradigms. As mentioned in the Introduction, juveniles that learn song from a live tutor copy song better than juveniles exposed to song from a tape tutor (Clay-Ton 1988, Beecher and Burt 2004, Derégnaucourt, Poirier et al. 2013). (A tape tutor as the name implies is simply passive playback of song to a bird.) If the cerebellum contributes to this socially-mediated aspect of song learning, then lesioning the cerebellum should impair learning. I propose a study with four groups: two tape-tutored groups where one receives sham lesions and the other receives bilateral lesions of CbL, and two live-tutored groups where again receives sham lesions and the other receives actual lesions of CbL. To determine whether CbL lesions specifically affect the socially-mediated aspects of song learning, I would measure similarity scores, a metric of how well a juvenile copies tutor song that is essentially the average correlation of song motif of a bird's song with the song motif of its tutor (Tchernichovski, Nottebohm et al. 2000, Mandelblat-Cerf and

Fee 2014). If similarity scores drop significantly in the live-tutored lesion group, and importantly do not drop significantly in the tape-tutored lesion group, it implies that the cerebellum is required specifically for socially-mediated improvement in song copying. On the other hand, if the cerebellum is involved with sensorimotor aspects of learning, then I would expect to see lower similarity scores in both lesion groups, the group that was tape-tutored and the group that was live-tutored, compared to the sham lesion groups. Pilot lesion studies that I carried out suggest that lesions of the cerebellar nuclei in adults do not grossly impair song production and also have no obvious effect on plasticity of song (Appendix V.5, Figure 38, Figure 39, Figure 40). Thus it would be particularly interesting if lesions before or during the development of song had an effect on song acquisition.

IV.2.B.9 Does the cerebellum co-ordinate dancing during song?

In this last section, I consider a possible function for the cerebellum that is not directly related to learning or producing song. Many species of songbirds including Bengalese finches perform dance-like movements when singing, especially when the song is directed to a conspecific. Video analysis finds that, like song and beak movements, dance-like movements of each bird are highly correlated with those of its tutor (Williams 2001). There are a few reasons I hypothesize the cerebellum might play a role in co-ordinating dance during song: (1) as noted, the cerebellum seems to be a central node in a pyramidal tract-like pathway, which largely interacts with ascending somatosensory and descending motor signals to and from the spinal cord; (2) previous anatomical studies of pigeon cerebellum suggest other major inputs to lateral lobule VI, the lobule that results showed projects to CbL, are from optical regions (Pakan and Wylie 2006), and the proposed

function of these pathways is to process optic flow (Wylie 2013) (movement of the visual scene across the eye that results from movement of the body); (3) a pathway from CbL through dorsal thalamus to Area X provides a way for processed optic flow to reach the song system where it might be needed during song. In this way, for example, the cerebellum could filter out optic flow that results from movements during the dance that accompanies song, allowing the bird to perceive local movement such as the response of a conspecific. If the cerebellum does perform a computation that cancels out self-generated movements during dance, then manipulating cerebellar output should affect dance. One approach would be to measure activity in cerebellar cortex and look for correlations between that activity and the kinematics of body movement. Activity in cerebellar cortex could be measured with multielectrode arrays or calcium sensitive dyes, and movement during dance could be measured with an accelerometer on the bird's head. A previous study used accelerometers similarly as a control (to show that an aversive white noise stimulus did not cause the bird to jump during song) (Gadagkar, Puzerey et al. 2016). There is previous work in support of the idea that cerebellar cortex represents kinematics of movement (Ebner, Hewitt et al. 2011). For these experiments I would specifically look for activity that correlates with kinematics of body movement in Purkinje cells of lateral lobule VI. I am not predicting that these correlations would only arise during the "dances" that accompany song. Rather I'm suggesting that what birds consider a "good" song performance might depend on body movement in a way that has not been recognized, and the cerebellum could be important for these "good" songs.

IV.2.C Functional considerations and conclusions

In closing, based on the data I have collected and the previous literature I have reviewed, I make predictions for the follow-up experiments I have proposed and draw conclusions about the functions of the novel projections in the song system that I have identified.

IV.2.C.1 Cerebellothalamic system

While the results regarding routes from the cerebellum to the song system were less clear, based on my findings in combination with the previous literature, I argue that the most probable role for the cerebellum is in beak movements or body movements, including body movements during song. The strongest evidence I found for a disynaptic pathway from CbN to the forebrain through DT_{CbN} was to an area of nidopallium. The iontophoretic viral injections in DT_{CbN} I proposed will help resolve the question of whether any subregions also send collaterals to Area X. If it proves to be the case that DT_{CbN} projects only to nidopallium and does not send collaterals to Area X, then perhaps the easiest next step in determining whether the cerebellum contributes to song would be to complete the follow-up studies on a possible cortico-cerebellar side loop in the pathways related to control of the jaw muscles and beak. Even if there is no strong anatomical evidence for disynaptic pathways through which the cerebellum interacts with the song system, it would still be worth simply looking at cerebellar activity during song, perhaps using multielectrode arrays. Establishing whether there is any correlation between cerebellar cortical activity and song would be another approach outside of anatomy to determine whether the cerebellum is involved with birdsong. While surprising, it could still be the case that birdsong is an exception to the rule that the cerebellum is required for learning and producing precisely coordinated motor skills.

IV.2.C.2 Thalamostriatal projections

I expect, given my data and previous studies, that thalamostriatal neurons in DLM and DT_{CbN} will have characteristics typical of "relay" neurons, similar to thalamostriatal neurons in mammalian intralaminar nucleus CL, and unlike the distinct class of thalamostriatal neurons found in the CM/Pf complex within the intralaminar nculei. Thus thalamostriatal neurons in DLM and DT_{CbN} will have a morphology typical of thalamic relay neurons, with bushy, highly branched dendrites, and a similar physiology, displaying LTS bursts under anesthesia. The collaterals of these neurons in Area X and medial striatum will have long processes with few branches. Varicosities will be obvious on these collaterals, that will prove to be *en passant* terminals that form preferentially on the dendritic spines of MSNs. EM studies will show that just like corticostriatal projections from LMAN and HVC, thalamostriatal terminals from DLM and DT_{CbN} will be close to dopaminergic terminals in the striatum. Slice studies will show that stimulation of thalamostriatal terminals yields large amplitude EPSCs in MSNs, suggesting that these inputs can drive MSNs during song. I further predict there will be no subcortical inputs to DLM. I predict that during song, a population

of MSNs is more likely to show correlated activity due to the input that population receives from *en passant* synapses of any individual thalamostriatal neuron in DLM. To summarize, I conclude that the main function of thalamostriatal projections from DLM to Area X will be to increase correlations between neural activity in Area X, and that this spatially-correlated variability in neural firing is required for variability in motor output.

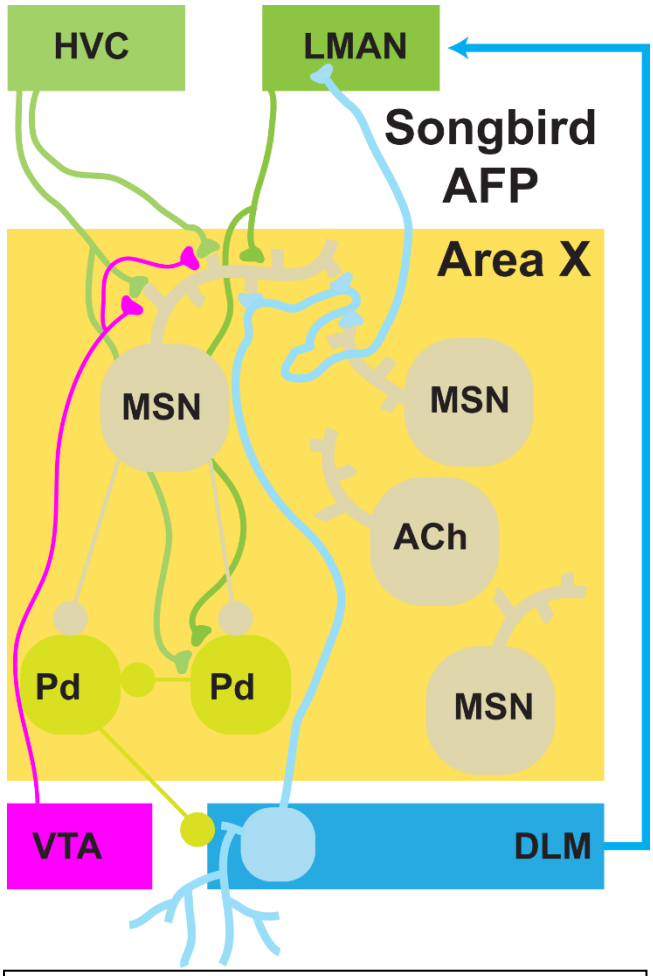

Figure 24. Predictions based on results and review of literature. I predict that thalamostriatal neurons in DLM (cyan neuron in blue box representing DLM) will have relay-like morphology with bushy dendritic trees and axons that form *en passant* terminals in Area X, preferentially targeting the spines of MSNs, but terminate in cortical song system nucleus LMAN.

V Appendices

V.1 Introduction

Results in the main text showed the cerebellar nuclei project to dorsal thalamus, and that dorsal thalamus projects to Area X and the medial striatum. The appendices include related results that may be relevant to future studies. **Full results for many of these studies can be seen in the supplementary information**: https://doi.org/10.6084/m9.figshare.5437975.

V.2 Afferents and efferents of the Bengalese finch cerebellar nuclei

V.2.A Comparison of projections of CbL and CbI to dorsal thalamus

Results showed that all regions of the cerebellar nuclei (CbN) project to dorsal thalamus (DT). Injections of dextran amines in DT produced retrograde label across CbN (Figure 17, Figure 18). The highest number of retrogradely labeled cells was in CbL but many were labeled in CbI and a few were labeled in CbM as well (Figure 17, Figure 18). This result raised the question of whether different regions in CbN project to different regions in DT. Targeting the different regions of CbN was difficult, but here I present results from one bird in which I injected tetramethylrhodamine-tagged dextrans in CbL and fluorescein-tagged dextrans in CbI (Figure 25). The clearest result in this animal was that anterograde signal in DT from the injection in CbI did not overlap completely with the signal from CbL (See for example, Figure 25I where signal from CbI, green, is close to DLM, while signal from CbL (magenta) is more ventral). It is difficult to draw conclusions from one case, but what is clear is that there is significant overlap of the projections of the two nuclei in the mediolateral plane; almost all sections of dorsal thalamus (Figure 25D-J) contain anterograde

label from both injection sites. Hence it is not the case that CbI and CbL target distinct regions of dorsal thalamus, and since CbL is the major source of the projections to dorsal thalamus, using anterograde label from CbL injections is a reasonable proxy for "DT_{CbN}" (the entire region of dorsal thalamus that receives cerebellar output).

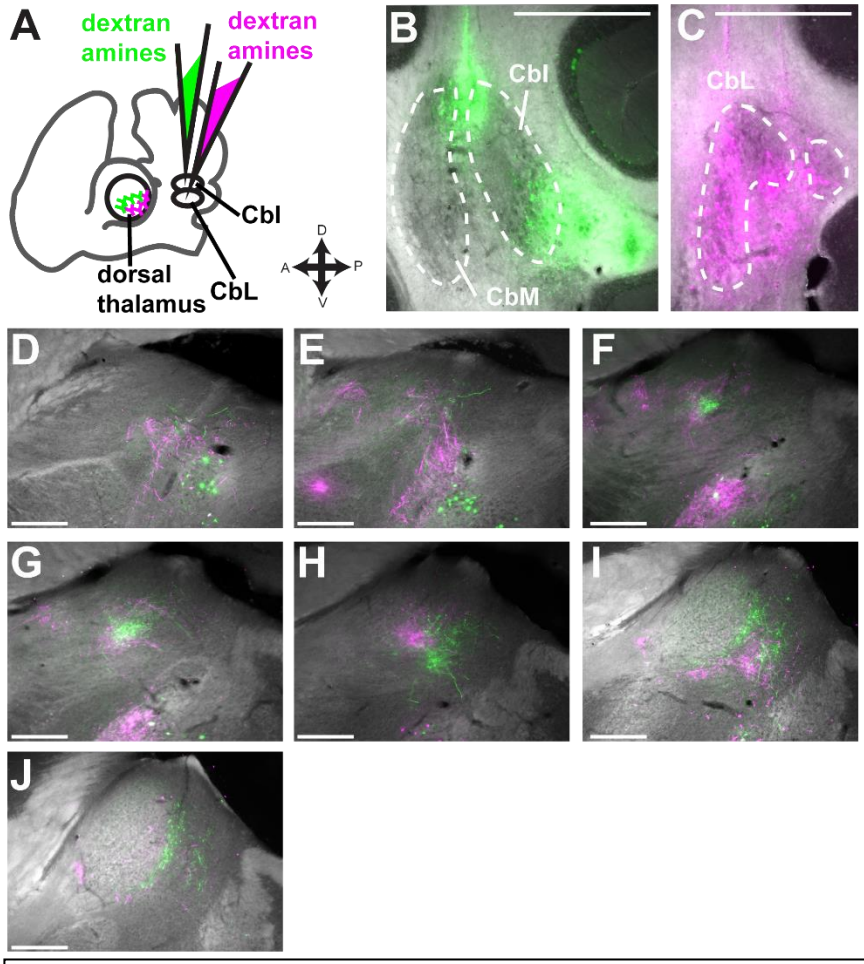

Figure 25. Projections of CbL and CbI to dorsal thalamus. **A,** schematic of experiment. **B,** injection site of fluorescein-tagged dextrans in CbI. **C,** injection site of tetramethylrhodamine-tagged dextrans in CbL. **D-J,** lateral to medial series of parasagittal sections showing anterograde label in dorsal thalamus from injections in **B** and **C**. All scale bars 500 µm.

V.2.B SpM sends collaterals to dorsal thalamus

As noted in the main text, injections in the CbN nuclei sometimes resulted in retrograde label of neurons in the medial spiriform nucleus (SpM), often both ipsilateral and contralateral to the injection. Thalamic nucleus SpM sends a massive projection to the cerebellar cortex (Karten and Finger 1976) whose passing fibers run parallel to the cerebellar peduncles leaving the cerebellar nuclei to enter the hindbrain. Hence it was not clear whether retrograde label of SpM arose due to uptake of tracer by passing fibers or because SpM also projects to the cerebellar nuclei. On the ipsilateral side from some injections in CbN there appeared to be retrograde label of in SpM and also label in dorsal thalamus, as if the label had traveled retrogradely from the injection in cerebellum to SpM, and then from there traveled anterograde to the collaterals that project to dorsal thalamus (see schematic, Figure 26A). Figure 26B presents an example showing the location of this label in dorsal thalamus and the morphology of these collaterals (Figure 26C). Notice that the collaterals are sparse, in contrast to the dense field of terminals that I considered anterograde label from CbL and CbI axon terminals (Figure 15, Figure 16, Figure 25). The example shown (Figure 26C) is actually the strongest case of label that appeared to be collaterals. Hence I am confident that the majority of the label contralateral to CbN injections, even when SpM was retrogradely labeled, was anterograde label of axon terminals and not label of SpM collaterals. For the entire series shown in figure 26C, please see the supplementary information at https://doi.org/10.6084/m9.figshare.5437975.

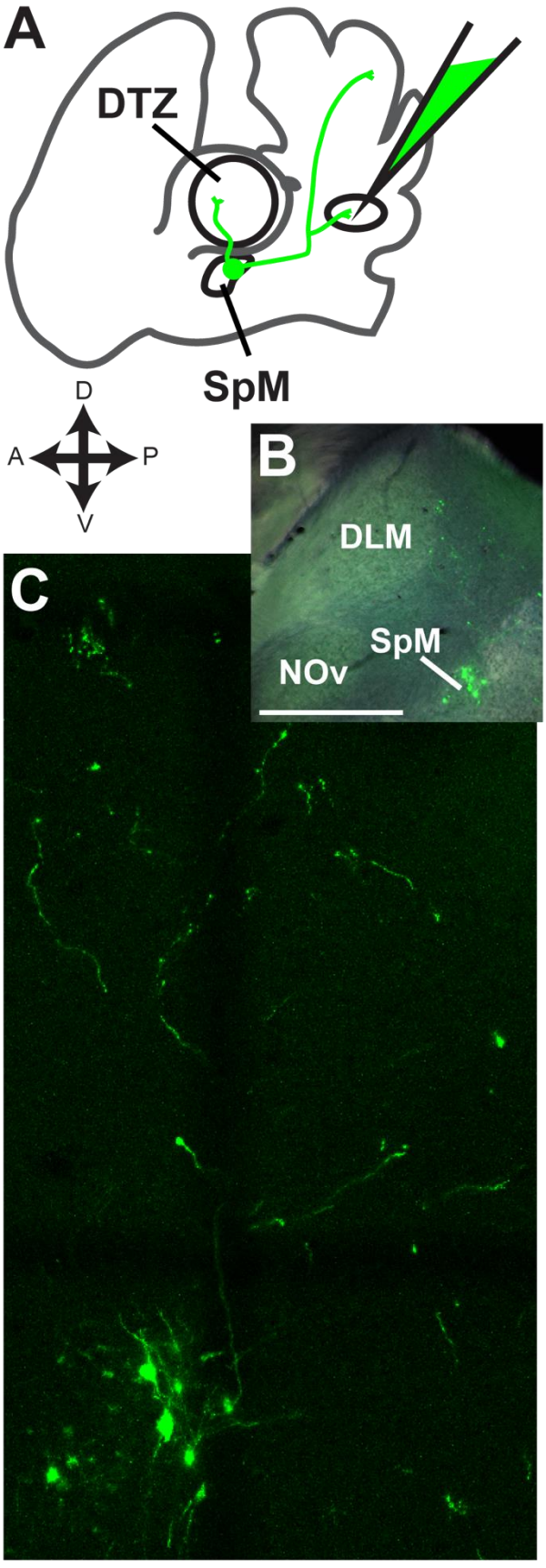

Figure 26. SpM may project to dorsal thalamus. **A,** schematic of experiment. **B,** retrograde label of SpM after injection of dextran amines in CbI. **C,** high-resolution confocal image of the same section shown in B, revealing that collaterals leave SpM and travel to dorsal thalamus **C**. All scale bars 500 µm.

V.2.C CbL projects to several other regions in the midbrain

As reported in the main text, CbL seems to be the major source of projections to dorsal thalamus, but injections in CbL also produced anterograde label throughout the midbrain and hindbrain. The other regions labeled were consistent across animals, and consistent with what has been reported previously for projections from the cerebellar nuclei in pigeons (Arends and Zeigler 1991). The major regions targeted were the red nucleus (Figure 27), the inferior colliculus (ICo) (Figure 28), and the principal precommisural (PPC) nucleus and SpM (Figure 29). Please see also the supplementary information at https://doi.org/10.6084/m9.figshare.5437975.

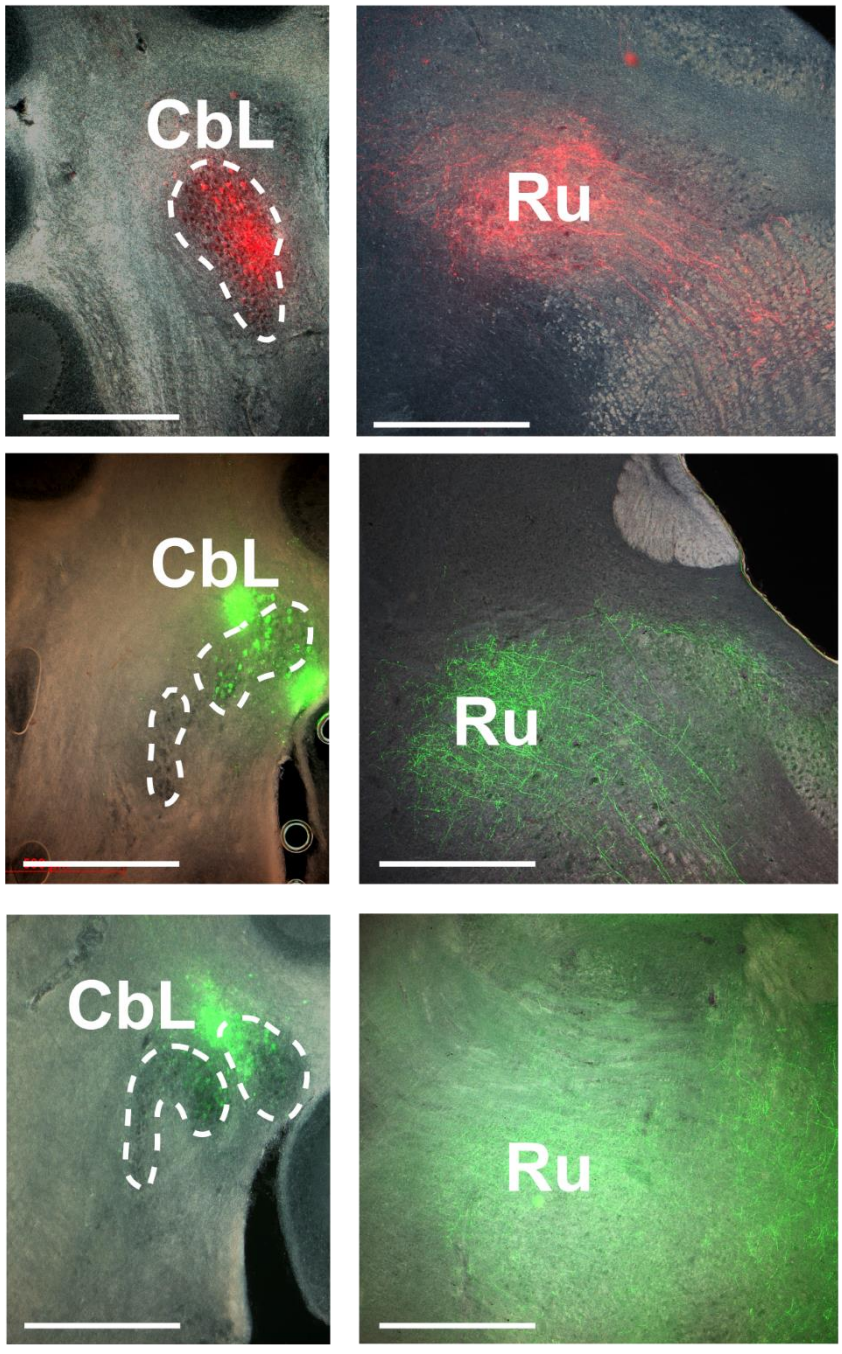

Figure 27. CbL targets the red nucleus.

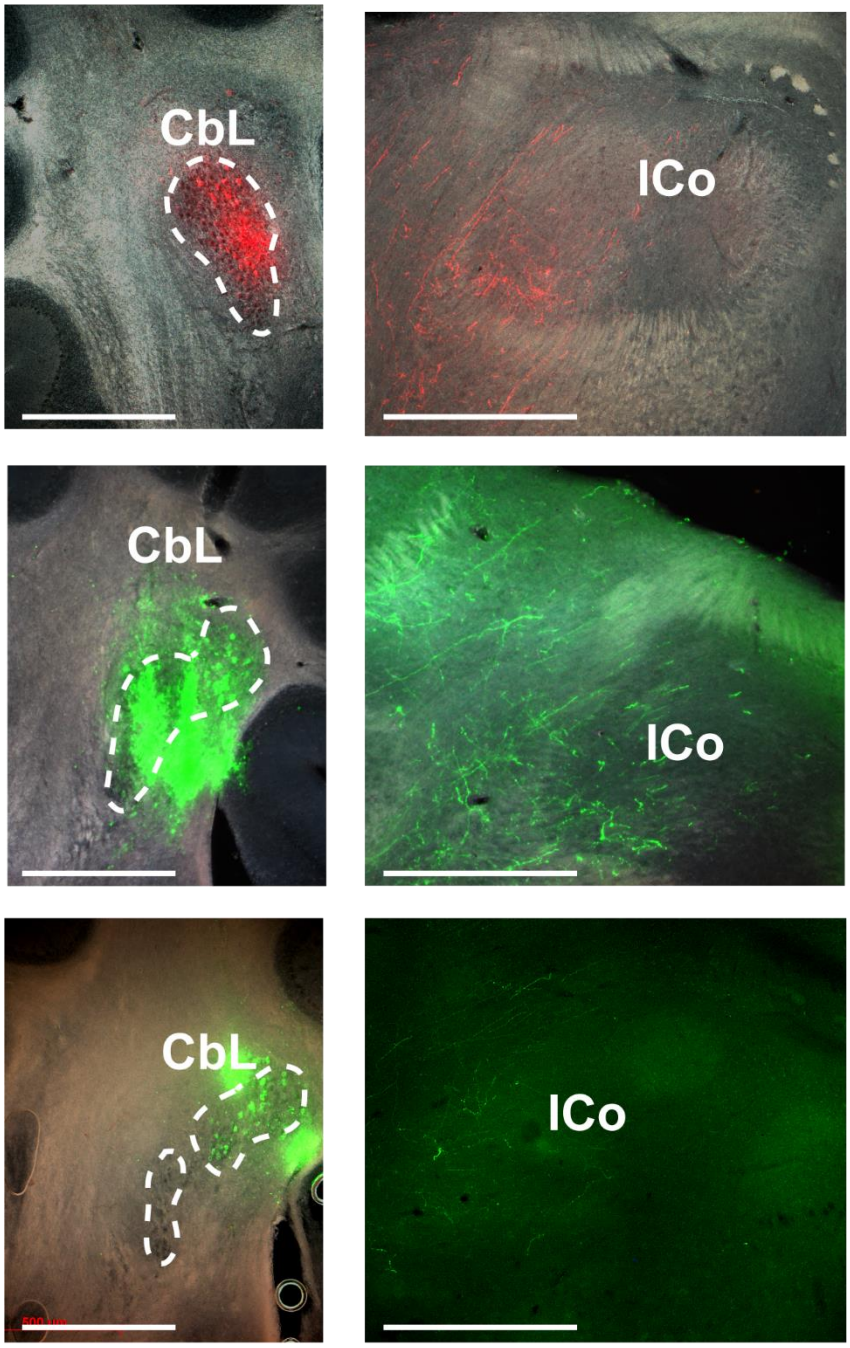

Figure 28. CbL targets inferior colliculus. (ICo)

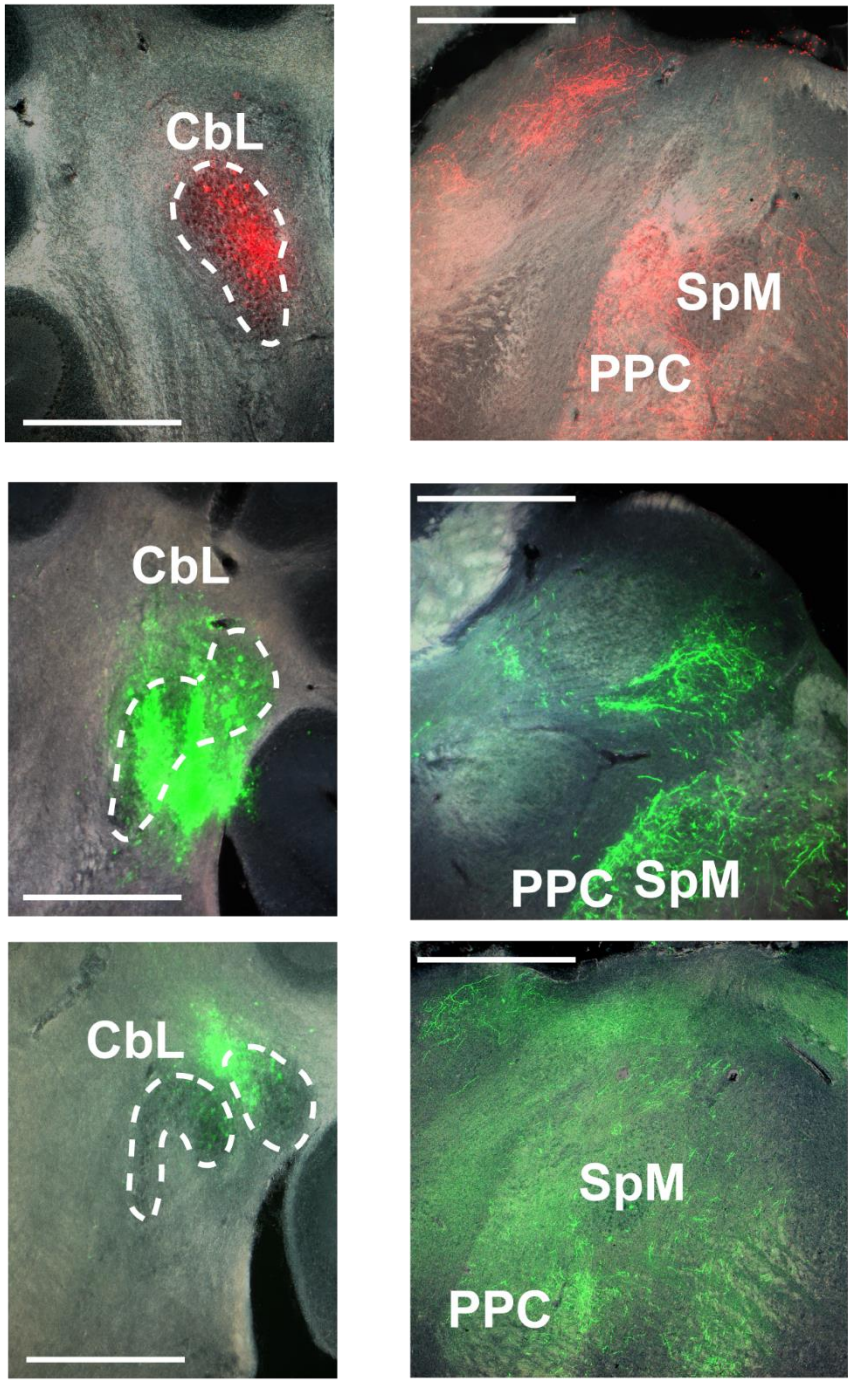

Figure 29. CbL targets SpM and PPC.

V.2.D Input from cerebellar folia to CbL

An obvious question, given the result that CbL projects to regions of dorsal thalamus that then project to Area X, is what regions in the cerebellar folia provide input to CbL. In the same birds where I mapped anterograde label of CbL, I took note of where Purkinje cells were retrogradely label by injections in CbL (Figure 30, n=3). I saw that the main lobules labeled were VI, VII, and VII (for example Figure 30C, white arrowheads. Dashed white box in Figure 30C is enlarged in Figure 30D). Occasionally I saw label of one or two Purkinje cells in other folia but this label was not consistent across animals. Hence I conclude that folia VI, VII, and VII are the main source of input to CbL. Although sections were cut parasagittal, I could see that the retrograde label of Purkinje cells was typically in the same mediolateral plane or slightly medial as CbL, i.e., it was in more lateral regions of the folia. This is also consistent with what was reported for pigeons (Arends and Zeigler 1991).

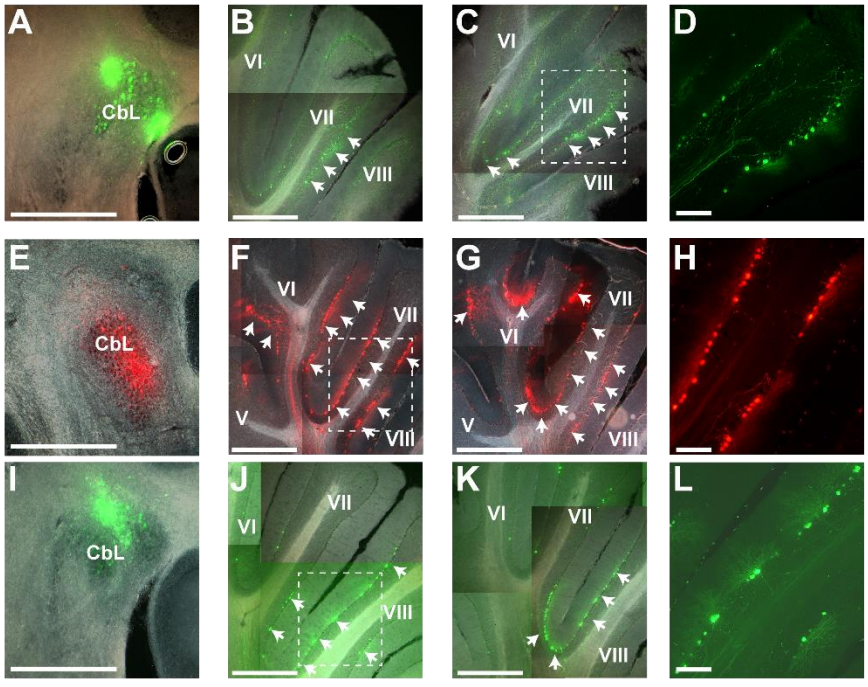

Figure 30. Inputs from cerebellar folia to CbL.

V.3 Regions of dorsal thalamus in Bengalese finches

V.3.A DLM as defined by projections from Area X

Dorsal thalamus (DT) has not been extensively studied in songbirds. Conclusions about what parts of DT project to Area X or MSt were based on results of tracing experiments executed in order to identify regions of DT based on their inputs. Experiments not reported in the main results were also carried out in an attempt to identify the regions of DT. First I show that song system nucleus DLM, as defined by input from Area X, is in fact well approximated by the heavily-myelinated area that appears as a "lighter" ball when viewed through a darkfield filter (Figure 31). Injections of GFP-expressing lentiviral vector (Figure 31A) or dextran amines (Figure 31H) always produced anterograde label of axons leaving Area X and label of the well-known large calyceal terminals (Gale, Person et al. 2008, Person, Gale et al. 2008) in DLM (for example Figure 31C) from striatopallidal neurons in Area X (n=3). This label of calyceal terminals in DLM was always confined to the "lighter" area identified as DLM using a darkfield filter (for example dashed white line in Figure 31F).

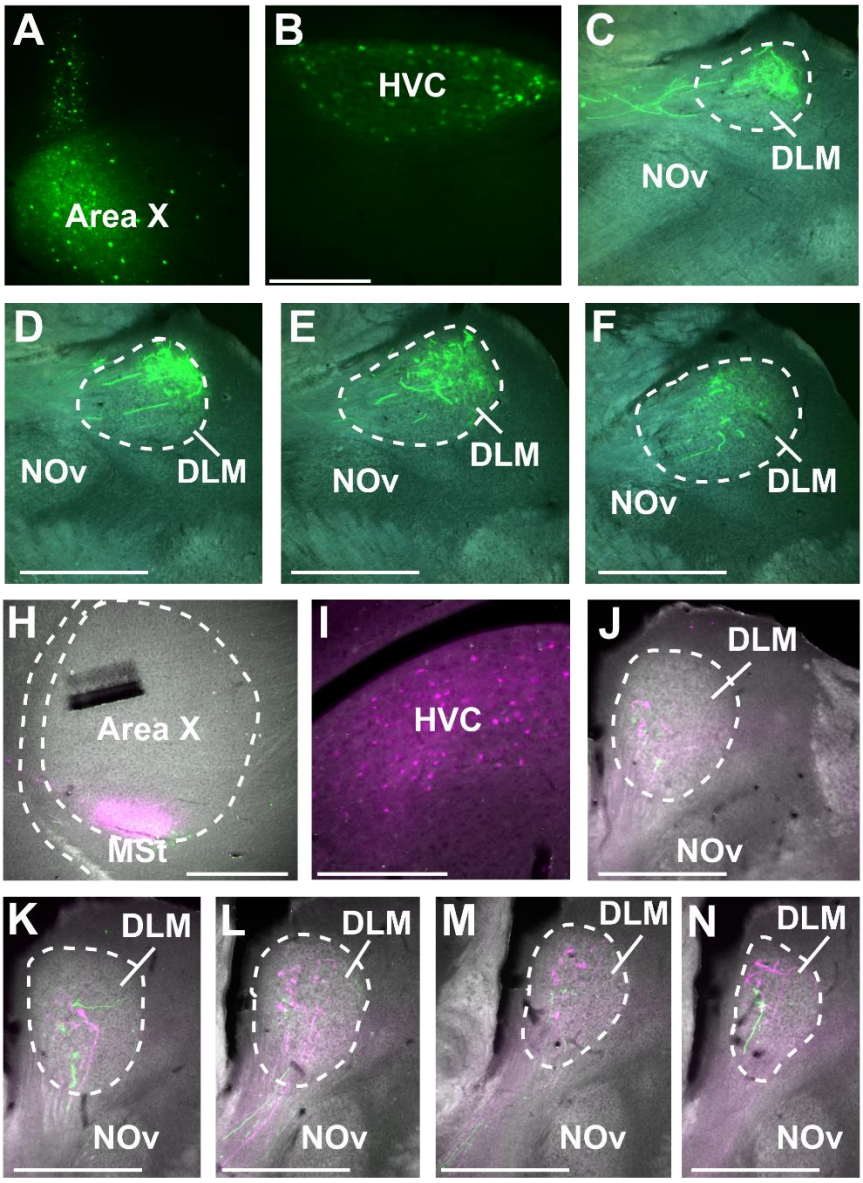

Figure 31. DLM as defined by projections from Area X. **A,** schematic of experiment. **B,** injection site of fluorescein-tagged dextrans in CbI. **C,** injection site of tetramethylrhodamine-tagged dextrans in CbL. **D-J,** lateral to medial series of parasagittal sections showing anterograde label in dorsal thalamus from injections in **B** and **C**. All scale bars 500 µm.

V.3.B DIP as defined by projections from globus pallidus

I also identified the region of dorsal thalamus that receives output from globus pallidus. This region was first identified in pigeons (Karten and Dubbeldam 1973, Kitt and Brauth 1982) and given the name dorsal intermediate posterior thalamus (DIP). It was important to identify this region in relation to cerebellar-recipient dorsal thalamus (DT_{Cb}) given that they are close to each other, and the goal was demonstrate which regions of dorsal thalamus project to Area X and the medial striatum. Before I attempted to see how DT_{Cb} and DIP relate to each other, I first made injections just in GP to verify whether results were similar to what is reported in other animals. Injections in GP yielded strong anterograde label across the lateral spiriform nucleus (SpL) (Figure 32D) and the subthalamic nucleus (STN) (Figure 32E). This is consistent with what is reported in pigeons (Medina and Reiner 1997) and in zebra finches (Person, Gale et al. 2008), so I was confident that I was able to target GP. Injections in GP also yielded label of a smaller region of dorsal thalamus that appeared ventral to DLM and anterior of DT_{Cb}. I take this area to be DIP. To demonstrate for certain where DIP sits in dorsal thalamus relative to DT_{Cb}, I made injections of dextran amines in both GP and in CbL, yielding anterograde label of axon terminals in the regions of DT that they target (n=2, Figure 32 presents results from one case). DIP occupies a region rostral of DT_{Cb} and caudal of NOv (for example in Figure 32H). Label in DT from GP injections extended medial of the label from CbL injections; i.e. DIP sits more medially in DT than DT_{CbN} (note DIP but not DT_{CbN} labeled in medialmost sections Figure 32K and Figure 32L), although there is a region where they overlap in the mediolateral plane (Figure 32G,H, and I).

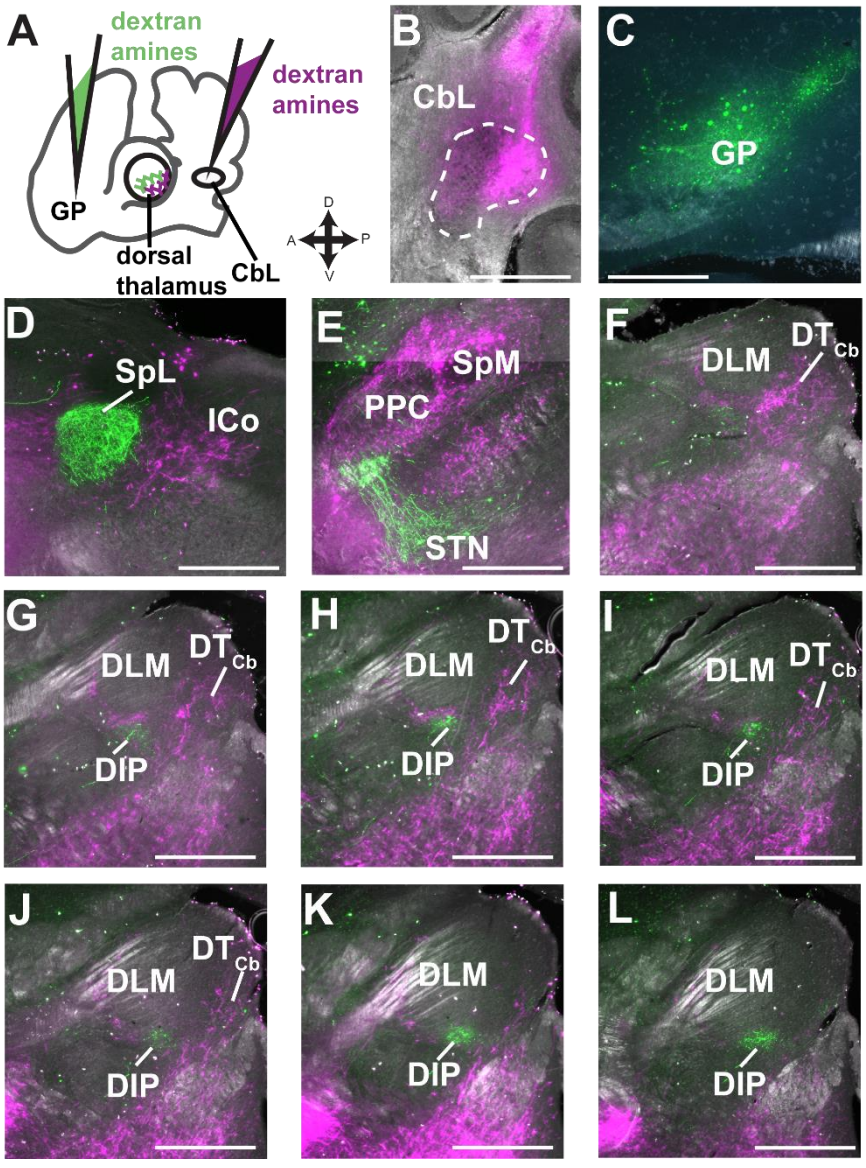

Figure 32. Globus pallidus projects to DIP, a region of dorsal thalamus anterior of DT(Cb) and ventral to DLM**A,** schematic of experiment. **B,** injection site of fluorescein-tagged dextrans in CbI. **C,** injection site of tetramethylrhodamine-tagged dextrans in CbL. **D-J,** lateral to medial series of parasagittal sections showing anterograde label in dorsal thalamus from injections in **B** and **C**. All scale bars 500 µm.

V.4 Relation of CbL projections to other song system nuclei in dorsal thalamus

V.4.A CbL axon terminals in some cases closely appose thalamic neurons retrogradely labeled by injections in Area X

Figure 33 presents results from one case where retrograde tracer was injected into Area X and anterograde tracer into CbI. Anterogradely-labeled cerebellar axons overlapped with retrogradely-labeled thalamic cell bodies in roughly the same mediolateral plane as songbird DLM, but posterior to it. This corresponded to DT_{CbN}. In no case did cerebellar axon terminals invade thalamic song system nucleus DLM.

However there are confounds associated with these results as acknowledged in the main text. Two possible explanations for retrograde labeling of cell bodies in DT_{CbN} after injections in Area X would be that (1) label was due to uptake of tracer by passing fibers, e.g., on their way to cortex. In addition, it might be the case that there are synapses in Area X, but these synapses do not take up enough tracer to strongly label the cell. Standard neuroanatomical tracers as analyzed with light or confocal microscopy do not make it possible to tell these two possibilities apart, because these tracers can travel retrograde as well as anterograde, and they do not specifically label synapses.

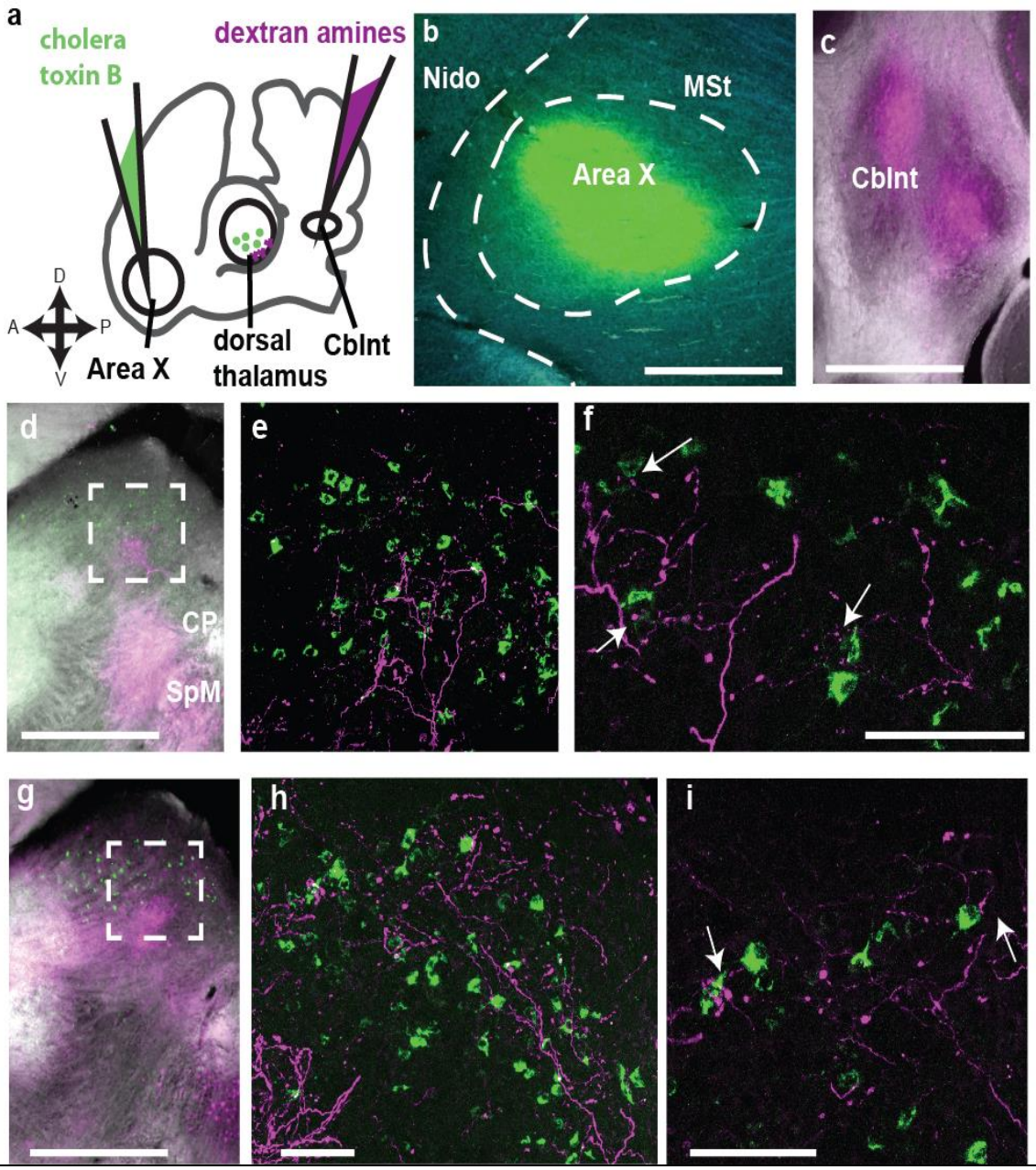

Figure 33. CbL axon terminals in some cases closely appose thalamic neurons retrogradely labeled by injections in Area X. **A,** Schematic representation of experiment, showing injection of retrograde tracer cholera toxin b in Area X, and injection of dextran amines in the contralateral cerebellar nuclei. **B,** Injection site in area X, the basal ganglia nucleus of the song system. Scale bar, 500 um. **C,** Injection site in the intermediate cerebellar nuclei (CbInt). Scale bar, 500 um. **D,** Site of overlap in dorsal thalamus, in the more lateral parts of the cerebellar-recipient region. Scale bar, 500 um. **E,** White box shown in d, z-projection of confocal image. **F,** Higher power image of z projection over a very small slice from a stack. White arrows: anterogradely-labeled cerebellar axons with terminal-like morphology closely apposed to proximal dendrites of thalamic neurons labeled by injections of retrograde tracer in Area X. **G-I**, Same as d-f, but in a section ~100 um more medial.

V.4.B CbL does not project to thalamic song system nucleus DMP

Hypothetically, output from the cerebellum could reach the song system via a disynaptic route from CbL through thalamic song system nucleus DMP to forebrain nucleus MMAN. To test this hypothesis, I injected dextran amines in CbL to anterogradely label axon terminals in DT, and I injected dextrans tagged with a different amine in MMAN to retrogradely label neurons in DMP (Figure 34A). Successfully targeting of MMAN produced anterograde label across HVC (Figure 34C)—HVC is a known target of MMAN. In only one case did I successfully target both MMAN and CbL (Figure 34B), and in that case retrogradely labeled neurons in DMP were on the dorsal surface of DT, whereas anterograde label of CbL terminals was in ventral DT (Figure 34I). Hence I found no evidence for a pathway from CbL to MMAN through DMP, and based on the very dorsal location of DMP (defined as retrograde label in dorsal thalamus from MMAN) in relation to the very ventral location of anterograde label in dorsal thalamus from CbL, it is unlikely that such a pathway exists.

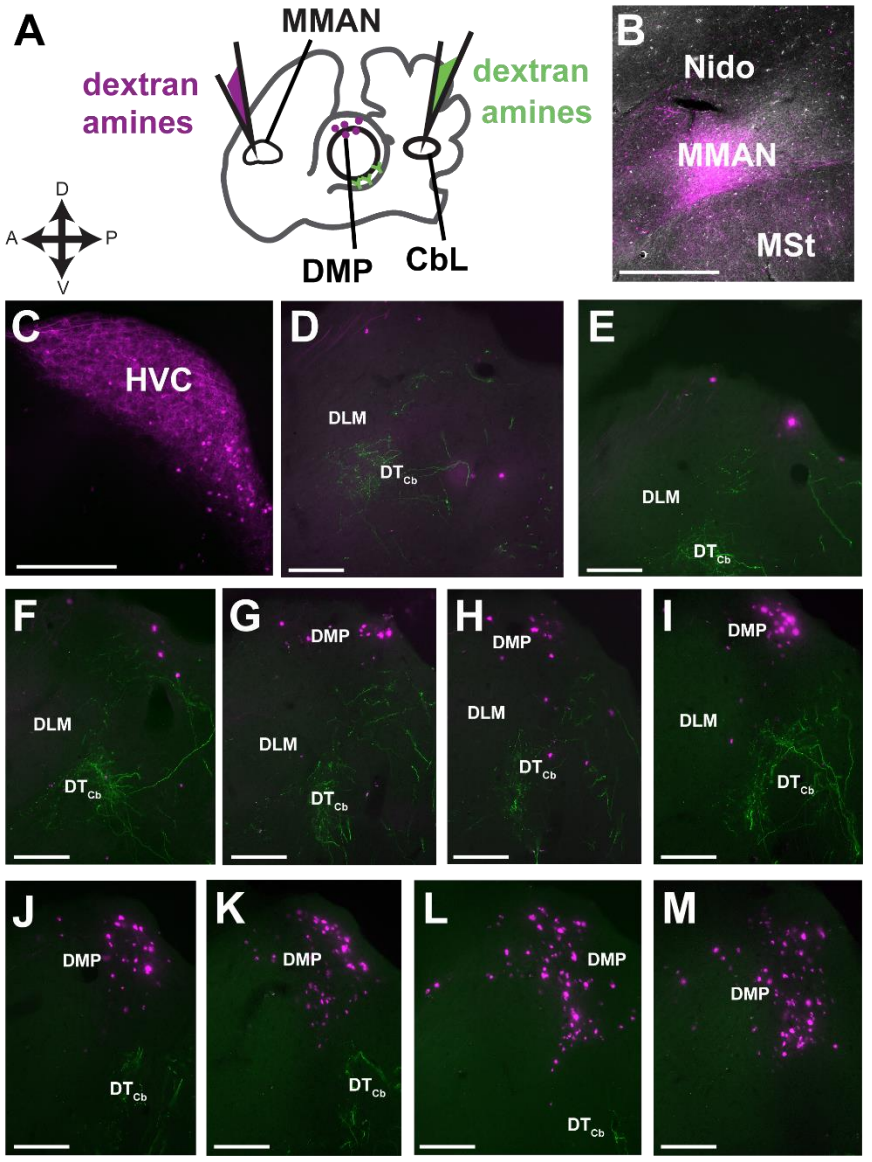

Figure 34. CbL does not project to DMP. **A,** schematic of experiment. **B,** injection site of tetramethylrhodamine-tagged dextrans in MMAN. **C,** anterograde label across HVC from injection in MMAN. **D-M,** lateral to medial series of parasagittal sections showing anterograde label in dorsal thalamus from injections in MMAN and CbL. Note DMP label is strongest near dorsal surface of thalamus while DT_{CbN} label is very ventral. All scale bars 500 µm.

V.4.C CbL does not project to thalamic song system nucleus Uva

I also tested whether output from the cerebellum could reach the song system via a disynaptic route from CbL through thalamic song system nucleus Uva to HVC. To test this hypothesis, I made injections of dextran amines in CbL to anterogradely label cerebellar axon terminals in thalamus, and made injections of dextrans tagged with a different amine in HVC to retrogradely label Uva (Figure 35. CbL does not project to Uva.A). While anterograde signal in DT from injections in CbL is close to Uva, the axons pass by it on their way to what appears to be a terminal field posterior to DLL ((Figure 35. CbL does not project to Uva.D, white arrow) based on comparison with the Karten zebra finch atlas (Karten, Brzozowska-Prechtl et al. 2013). Because of its shape, which is obvious even in unstained tissue, I could also identify Uva in other birds where I made CbL injections (e.g. the injections in Figure 15and Figure 16) and never saw any evidence that CbL axons terminate in Uva.

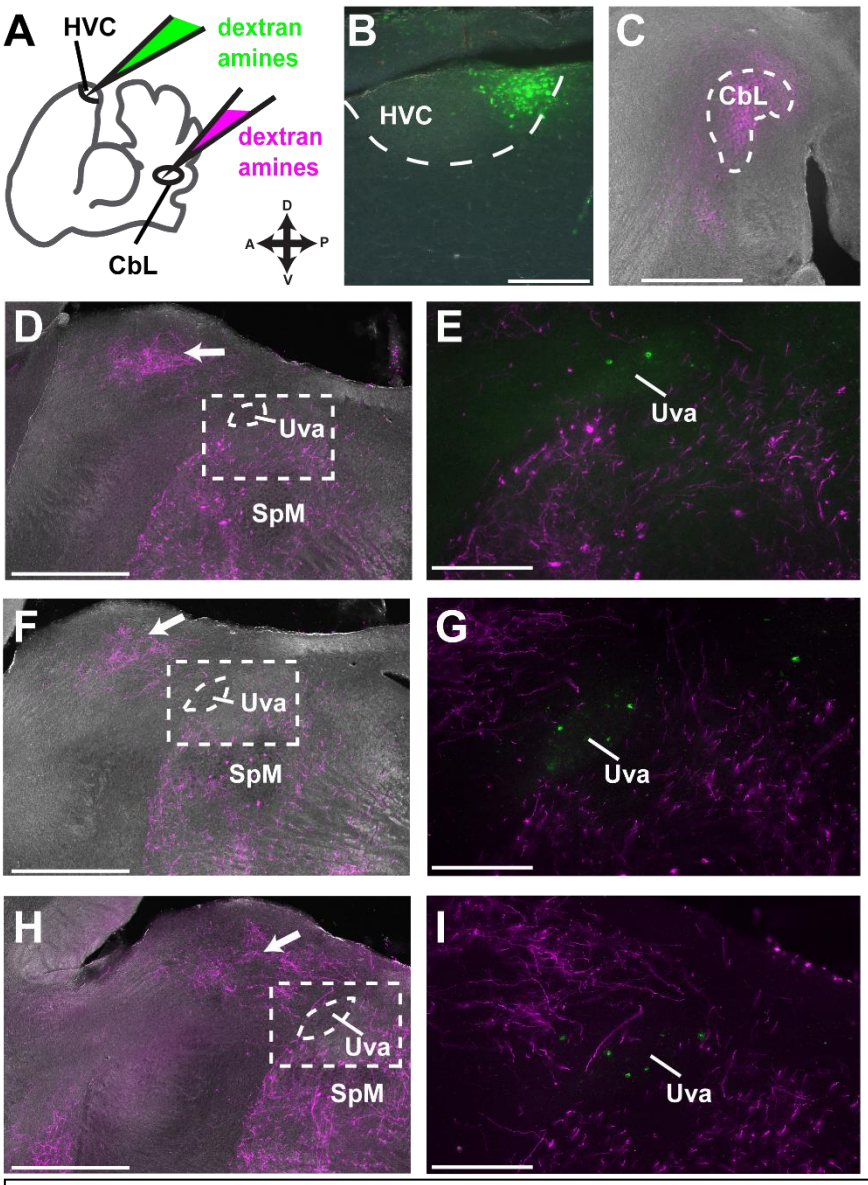

Figure 35. CbL does not project to Uva. **A,** schematic of experiment. **B,** injection site of fluorescein-tagged dextrans in HVC. **C,** injection site of tetramethylrhodamine-tagged dextrans in CbK. **D, F, H,** lateral to medial series of parasagittal sections showing retrograde label in Uva from injection in **B** and anterograde label from injection in **C**. Same sections are shown in **D, F, H** but at higher power and without DIC filter. Note that anterograde CbL label is not near Uva as defined by retrograde label. All scale bars 500 µm.

V.4.D CbN axon terminals closely appose dendrites and soma of neurons in dorsal thalamus that project to nidopallium

Anterograde results from lentiviral injections in dorsal thalamus suggested that DT_{Cb} projects to nidopallium. I tested the hypothesis that CbL output reaches nidopallium through dorsal thalamus (Figure 36A) by injecting dextran amines in nidopallium to retrogradely label neurons in DT (Figure 36B) and also injecting dextran amines in CbL (Figure 36C) to label exon terminals in DT.

These injections did yield areas of overlap of retrograde and anterograde label in lateral DT, in the region posterior to DLL (Figure 36D, white arrow). I did find positive evidence (n=1) that CbL axons closely appose the dendrites of thalamic neurons that project to nidopallium (Figure 36E, black arrow with white outline. This image is a high-resolution confocal scan of the section shown in Figure 36D). The evidence from this one case does support the idea that CbL output reaches nidopallium anterior of LMAN through DT_{CbN}.

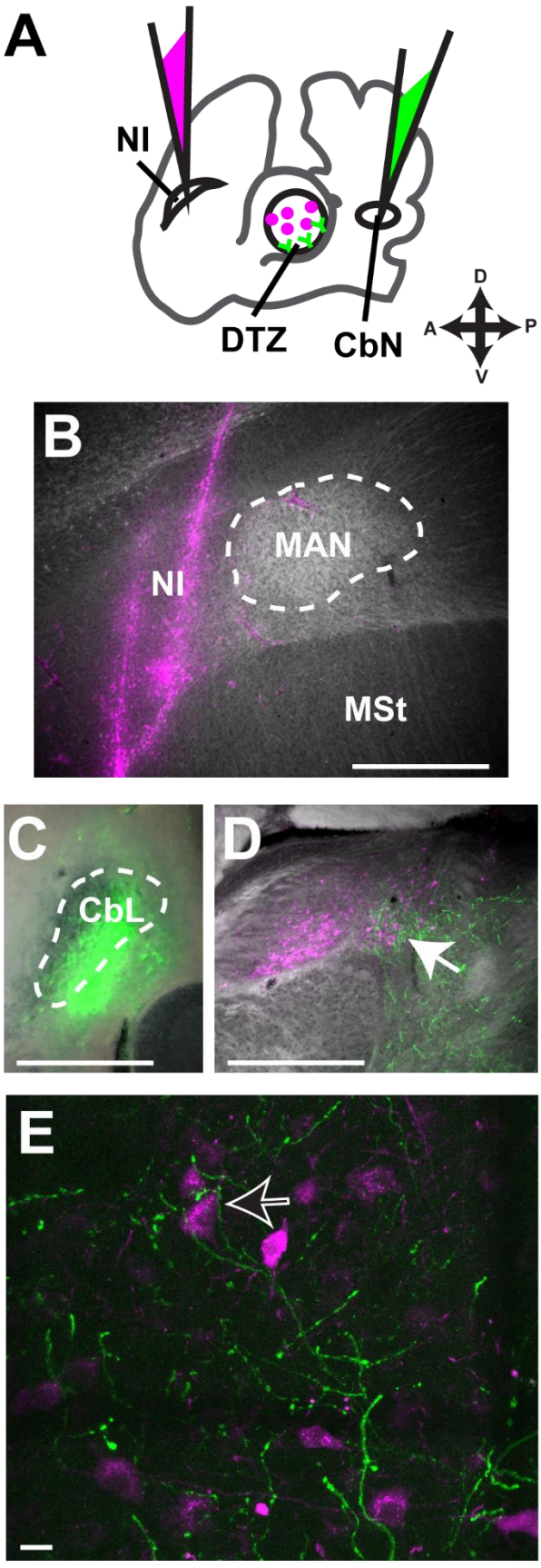

Figure 36. Output from CbL can reach cortex through dorsal thalamus. **A,** schematic of experiment. **B,** injection site of tetramethylrhodamine-tagged dextrans in nidopallium anterior of LMAN **C,** injection site of fluorescein-tagged dextrans in CbI.. **D-J,** example section in lateral dorsal thalamus showing label in dorsal thalamus from injections in **B** and **C**. White arrow indicates region imaged with confocal in **E**, where black arrow indicates axon terminals closely apposed to soma of thalamic neuron that projects to nidopallium All scale bars 500 µm.

V.4.E Lentiviral vector injections in DIVA labeled HA

Injections in more lateral regions of dorsal thalamus (the area shown in Figure 37) did not yield synaptophysin-GFP signal in the medial striatum. Instead, label was found in caudal nidopallium overlying the globus pallidus, or in hyperpallium at the rostral pole of the brain. Presumably this area is the region named HA by Wild (Wild 1997, Wild and Williams 2000), implying the thalamic injection site included the somatosensory nucleus in dorsal thalamus DIVA.

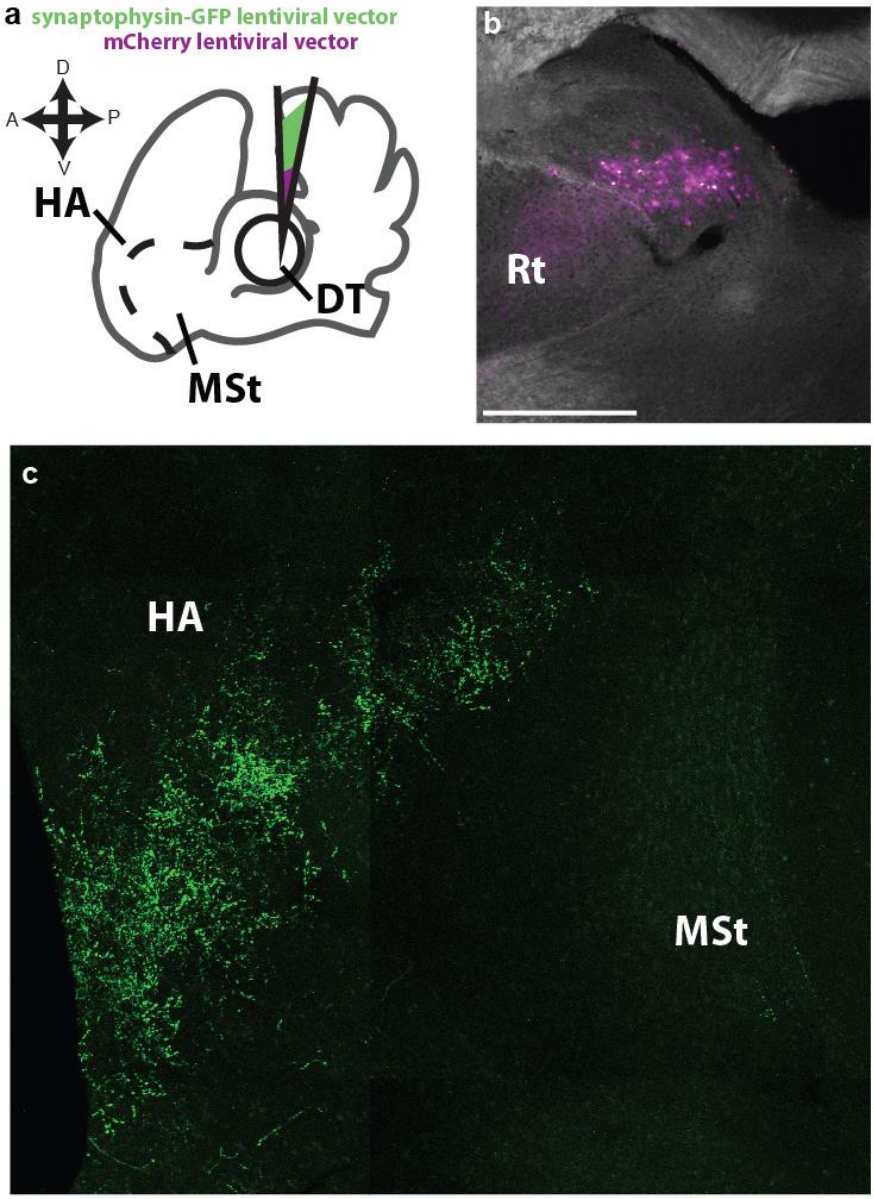

Figure 37. Synaptophysin-GFP label in HA from dorsal thalamic viral injection site that included DIVA. **A,** schematic of experiment. **B,** injection site of lentiviral vector in dorsal thalamus. **C,** synaptophysin-GFP label in HA, suggesting that injection mainly included thalamic nucleus DIVA. All scale bars 500 µm.

V.4.F Discussion of additional anatomical results

This section contains results related to the main findings in this study. I discuss their relevance. Results from injections in CbI and CbL implied that CbI may target different subregions of DT than CbL, although of course it should be confirmed by reproducing the result in more than one animal. If the result does hold up, another interpretation would be that the division of the nuclei into CbL and CbI—which was originally based solely on cytology—may not be appropriate, since they both project to dorsal thalamus. It would be appropriate to consider them separate if they have separate inputs or the regions they project to in dorsal thalamus have distinct targets.

I also provided some positive evidence that SpM may send collaterals to DT. This is interesting given that SpM is a major source of input to the cerebellum, and DT is one important target of output from the cerebellum. I note that the region of DT that receives SpM collaterals appears to include DT_{CbN} (although I did not test this). If it does include DT_{Cb}, then DT_{Cb} may receive an "efference copy" of signals that SpM sends to the cerebellum, and neurons in DT_{Cb} would then integrate this "efference copy" with output from the cerebellum.

In addition I identified the folia that send their input to CbL. Based on retrograde labeling of Purkinje cells in the folia from tracer injections in CbL, I concluded that folia VI, VII, and VIII are its main sources of input. Interestingly, retrograde tracer studies with CtB have shown that these folia themselves are the target of SpM as well as the pontine nuclei and several brainstem and midbrain nuclei related to visual pathways (Pakan and Wylie 2006). Obviously birds depend heavily on visual feedback for behavior like flight. Many songbirds like the Bengalese finch

perform a “dance” during song that presumably requires integration of visual feedback; the cerebellum could be one possible source of visual feedback for the song system during song.

The results here also addressed several questions about subregions of dorsal thalamus. I confirmed the location of thalamic song system nucleus DLM in Bengalese finches by making injections in Area X. I then showed that DIP, the target of globus pallidus output in dorsal thalamus, is a region ventral to DLM and anterior to DT_{Cb}. The fact that DIP is ventral to DLM in Bengalese finches, and like DT_{Cb} appears “crowded out” by DLM, is consistent with the idea that DLM like other song system nuclei arose from duplication of the existing motor pathways (Feenders, Liedvogel et al. 2008).

Lastly I tested three hypothetical disynaptic pathways from CbL to the forebrain through dorsal thalamus. I did not find evidence that CbL axons terminate in DMP or Uva, so I consider it unlikely that CbL output could reach MMAN or HVC (respectively) through dorsal thalamus. I did show that CbL may communicate with nidopallium outside the song system through dorsal thalamus. This result is based on only one case, but if it held true when the experiment is replicated, it would raise an interesting question that has implications for song system function. The question it raises is whether single neurons in dorsal thalamus project to both Area X and nidopallium outside of LMAN. Retrograde tracing studies in mammals suggest that a sparse population of thalamic neuron projects to both striatum and cortex, but that the majority of neurons in the parafascicular nucleus (PF), the main source of the thalamostriatal system, project only to striatum.

V.5 Possible cerebellar contributions to song

V.5.A Electrolytic lesions of CbL do not produce gross distortions of song

I tested whether CbL contributes to song by creating electrolytic lesions in the nucleus (n=2). The lesions did reduce the size of CbL (Table 3, Figure 38B) presumably due to cell death as implied by gliosis at the site of lesions (Figure 38B, black arrows) but did not have any obvious effects on the birds' ability to produce song (Figure 38D, F).

Table 3. Electrolytic lesions of CbL

	CbL size after lesion (normalized)	
Bird ID	left hemisphere	right hemisphere
bird 1 (ID: gy6or6)	0.83	0.97
bird 2 (ID: bl26lb16)	0.59	0.76

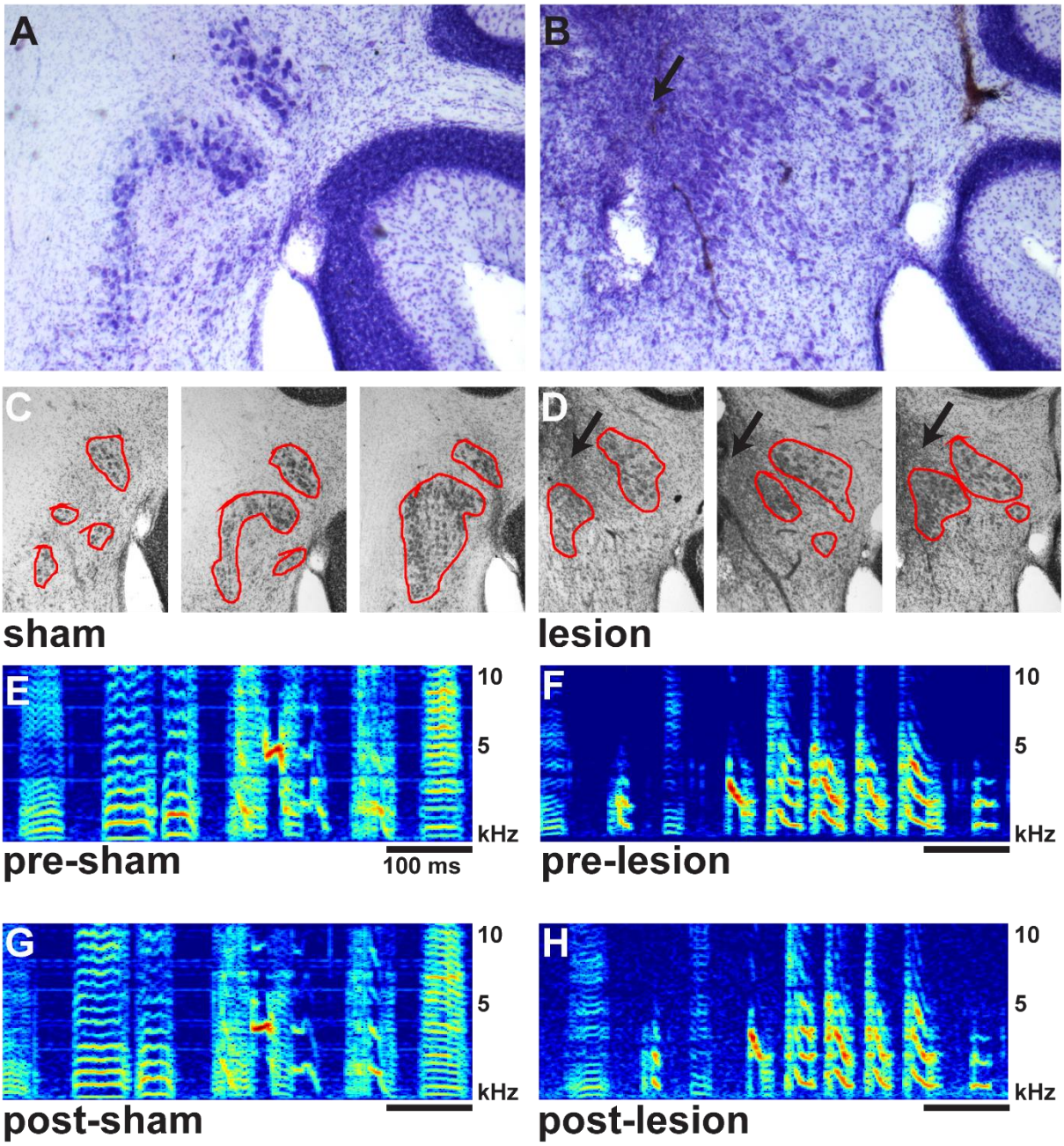

Figure 38. Electrolytic lesions of CbL do not grossly impair song. **A,** Example section of CbL without lesions. **B,** example section of CbL with electrolytic lesion, in same mediolateral plane as section A. Black arrow denotes gliosis at site of lesion. **C,** CbL in sham surgery bird. Red outlines are Regions of Interest used to estimate area of CbL **D,** CbL in another bird after electrolytic lesions. **C,** song of sham bird before surgery. Black arrows denote gliosis at site of lesion.**D,** song of lesion bird before surgery. **E,** song of sham bird after surgery. **F,** song of lesion bird after surgery.

V.5.B Electrolytic lesions of CbL can have long term effects on acoustic parameters

In one case I did find that amplitude dropped significantly after lesions of CbL (Figure 39). I did not see this effect in a sham bird, but I also did not see the effect in the other bird with CbL lesions ("bird 2") in spite of the fact that more of the nucleus was lesioned in this second bird (Table 3).

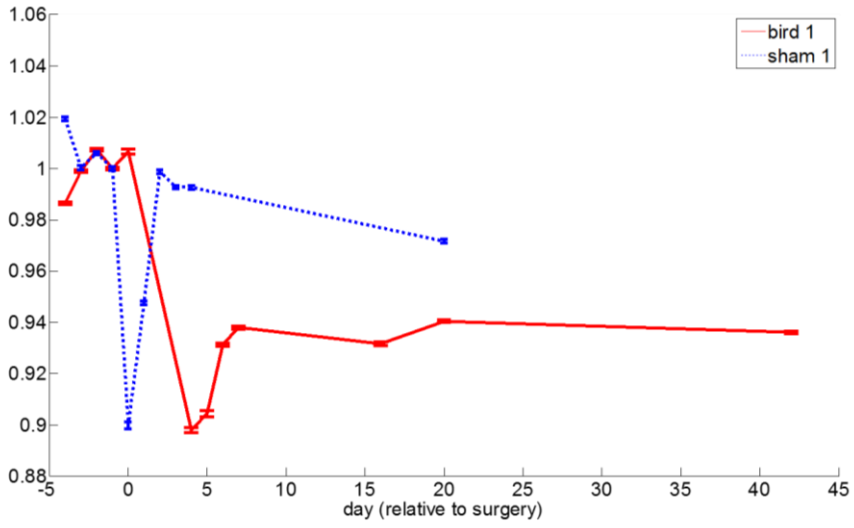

Figure 39. Electrolytic lesions of CbL can have long term effects on acoustic parameters. Lesion bird (red line) showed a significant drop in average amplitude of all syllables compared to sham bird (blue dashed line). There was no obvious reason why the sham bird's song had a large drop in amplitude the day of surgery but it may be related to there simply being less songs recorded that day (and hence a greater chance for the normalized difference to be farther from the mean).

V.5.C Electrolytic lesions of CbL do not impair the ability to change song in response to aversive reinforcement experiments

Lastly I carried out behavioral experiments in which syllables birds sang were targeted by a computer and singing was punished with aversive white noise when the pitch fell below a certain threshold (as described in the Introduction, section I.2.A.3.e, and in the additional methods, section V.6.E). This paradigm has been shown to induce a change in the distribution of sung pitches previously (Charlesworth, Tumer et al. 2011). I tested whether birds could shift the distribution of pitches they sang before and after CbL lesions. Results did not show any obvious difference in birds' ability to change their song in this paradigm after lesions (Figure 40A and B). Results shown here are for bird 1 but were similar for bird 2. There was no obvious impairment of learning.

V.5.D Discussion of results from electrolytic lesions of CbL

There are two reasons why no clear result emerged from these pilot lesion studies. Quantification of lesion size suggest that the lesions were not complete as shown in Table 3. It is also difficult to detect whether there was an effect of lesions on behavior from one animal. Future studies could make more complete lesions could be made by lesioning a multiple dorsal-ventral co-ordinates. It would be advisable to make neurotoxic lesions, e.g. with ibotenic acid, to also avoid a passing fibers confound. Obviously future studies should also include more than one animal. Previous studies that have shown have lesions impair song plasticity in this paradigm have reported differences based on results pre- and post-lesion across multiple animals (Hoffmann, Saravanan et al. 2016).

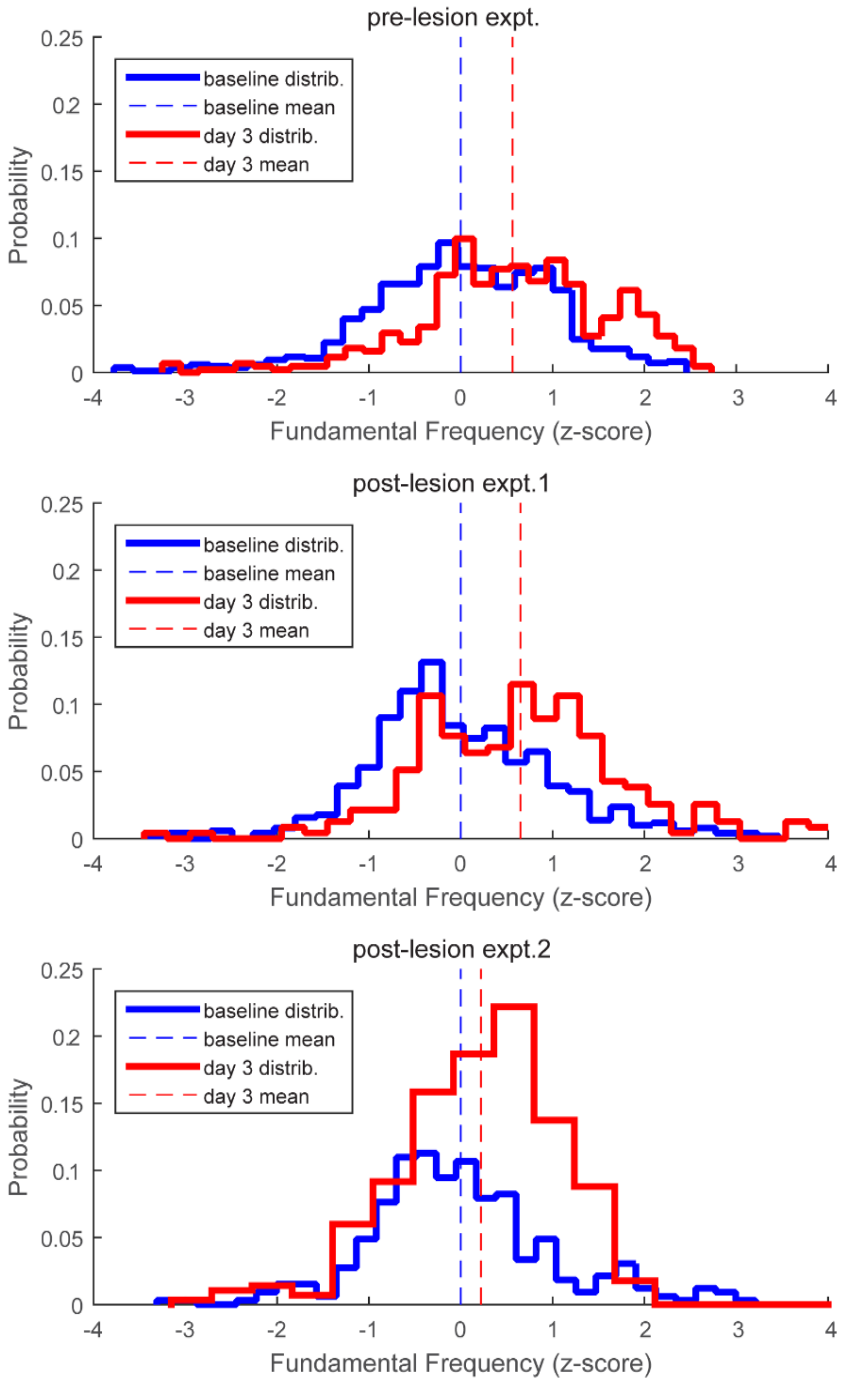

Figure 40. Electrolytic lesions do not impair ability to change song in aversive reinforcement experiments. Results from three white noise experiments showing baseline distribution (blue) and distribution after three days of white noise targeted at bottom 75% of distribution (red). The top panel shows results from an experiment carried out before CbL lesions. The bottom two panels show results from two white noise experiments carried out after CbL lesions. There was no obvious impairment of the bird's ability to modify song in response to white noise.

V.6 Additional methods

This section describes methods for experiments in the appendices

V.6.A Additional stereotactic co-ordinates and tracer injection methods

Table 1. Stereotaxic co-ordinates **Target**	**Anterior of y0**	**Lateral of y0**	**Depth**	**Beak bar angle below horizontal**
Area X	5.5-5.7	0.9-2.2	2.9-3.1	20
Globus Pallidus	0.7	2.00	2.95-3.75	64
Globus Pallidus	0.9	2.35	3.6-3.75	64
MMAN	5.35	0.35	2.25	45
HVC	0.0-0.5	2.3	0.25-0.4	45
Nidopallium anterior of LMAN	5.4 –5.6	1.5–1.1	2.4–2.6	45

All injections were made with iontophoresis of dextran amines, as described in the main methods, except where noted in Appendices. Cholera toxin B conjugated with fluorophores (1% in 0.2M phosphate buffer) (Life/Invitrogen) was also injected via iontophoresis as described in the main methods. Fluorogold (2% in 0.9% saline) was injected with Nanoject II as described in the main methods. Concentrations higher than 0.5% (weight/volume) and iontophoretic injections of any concetration were found to be necrotoxic at the site of injection in Area X and in dorsal thalamus. Note that two sets of co-ordinates are given for globus pallidus because its shape changes moving from lateral to medial, the more medial region has a longer extent in the dorsoventral plane.

V.6.B Electroylytic lesions of CbL

Lesions were targeted with stereotaxic surgical device. Co-ordinates were similar to those described for CbL in main methods. Electrolytic lesions were made with 100 kOhm platinum-tungsten electrodes (Microprobes) and a Model 2100 A&M stimulator. At each co-ordinate, positive current (50 µA) was passed for 1m. Afterwards the electrode was left undisturbed for 2m. This lesion procedure was repeated twice at one site in each hemisphere to increase the size of the lesions.

V.6.C Histology and estimate of size of CbL lesions

At the end of behavioral experiments, CbL lesion birds were sacrificed and perfused as described in the main methods. Brains were cut parasagitally into 50um sections with a sliding freezing microtome again as described in the main methods. Sections containing the cerebellum were mounted and then a Nissl stain was performed on the sections with cresyl violet. Three brains from birds that had not undergone electrolytic lesion experiments were sectioned and stained in the same manner. The size of CbL lesions was estimated using measuring tools in ImageJ as follows: in all birds, including unlesioned birds, three consecutive sections were chosen that contained CbL. Across all birds, the number of sections containing CbN did not vary by more than one section per bird, and it was easy to align the series from each bird by eye. (By align, I mean arrange the sections from each series in order next to each other, forming a matrix where each column is a series from one hemisphere and each row should be the same mediolateral plane across hemispheres). In series from the experimental group, sections without lesions were used to help alignment with series from unlesioned birds, and thus ensure that the same three sections from each series were used

across all animals. In each of the three sections from the unlesioned birds, a line was draw around CbN to create a "region of interest" (ROI) that can be saved along with the image in ImageJ. The area of the ROI from each section (in pixels) was obtained with ImageJ and then summed. In this way an estimate of the size of CbL from six hemispheres in three unlesioned birds was obtained. The average of these six measurements was taken to be the average unlesioned size of CbL The same procedure was repeated for the three sections from each hemisphere for each lesioned bird. The sum of area of remaining CbN after lesions for each bird was divided by the average size of unlesioned CbN to obtain a normalized measure of the area remaining after lesions. A separate measure was obtained for each hemisphere in the lesioned birds.

V.6.D Measurement of effect of CbL lesions on acoustic parameters

To determine whether CbL lesions affected song, acoustic parameters of song were measured as follows: each bird's song consisted of approximately 5-10 elements known as syllables, and these elements were labeled from bouts of song by hand using a GUI. All song bouts from three days before the lesions and for several days after were labeled. The MATLAB scripts measured each acoustic parameter from a fixed time point from each rendition of each syllable. The following acoustic parameters were measured for each syllable: pitch, duration, amplitude, spectral entropy, and high-low ratio. Pitch was defined as the peak in the power spectrum between two frequencies chosen for the analysis. This frequency band was chosen so that it captured a high-amplitude harmonic across renditions of the syllable. Amplitude was measured in arbitrary units as the summed spectral density in a brief time window centered on the point at which it was measured. Spectral entropy was calculated as $-\sum p_i \log_2(p_i)$, where p_i is the power in frequency bin i from

the spectrum at the time point of interest. This measure serves as a proxy for how pitchy (low entropy) a syllable is. The high-low ratio was simply the summed power spectral density for frequencies below 5 kHz divided by the same sum for frequencies above 5 kHz. In Bengalese finch song, "pitchy" high-amplitude syllables typically also have higher power in the frequency range above 5 kHz than other syllables. "Delta" measures of these parameters were also taken, by measuring them at 20% and 80% of the total duration of the syllable and then taking the difference of these two measurements.

After these measurements were taken from all renditions of all syllables, each was normalized to the average value from the three days of baseline song preceding lesion surgery. No obvious changes in any acoustic parameters were seen except for the drop in average amplitude for one bird shown in section V.5.A.

V.6.E Aversive reinforcement experiments

These experiments followed the procedure described in (Tumer and Brainard 2007). Briefly: computer software monitored the birds' song in real time and delivered an aversive blast of white noise when the song met certain criteria. To do so, the software compared brief segments of song to a template. The first criterion is that there is a good enough match between song and template, where "good enough" is a value set by the user. When the match was good enough, the software then estimated the pitch, i.e. the peak in the power spectrum within a user-specified frequency band. The second criteria is that the pitch fall within a smaller "punished" band. When the estimated pitch fell within the "punished" band, it triggered a blast of white noise.

For the experiments shown in Figure 40, the threshold below which sung pitches were punished with white noise was set at the sixtieth percentile of the distribution estimated from the last twenty songs sung on the previous day. This distribution was estimated for each day and the threshold adjusted accordingly for all three days during which white noise was delivered. The pitch of syllables sung that were punished was measured from catch trials. 10% of all trials were catch trials.

VI References

Albin, R. L., A. B. Young and J. B. Penney (1989). "The functional anatomy of basal ganglia disorders." Trends in neurosciences **12**(10): 366-375.

Alexander, G. E., M. R. DeLong and P. L. Strick (1986). "Parallel organization of functionally segregated circuits linking basal ganglia and cortex." Annual review of neuroscience **9**(1): 357-381.

Ali, F., T. M. Otchy, C. Pehlevan, A. L. Fantana, Y. Burak and B. P. Ölveczky (2013). "The basal ganglia is necessary for learning spectral, but not temporal, features of birdsong." Neuron **80**(2): 494-506.

Alloway, K. D., J. B. Smith and G. D. R. Watson (2014). "Thalamostriatal projections from the medial posterior and parafascicular nuclei have distinct topographic and physiologic properties." Journal of Neurophysiology **111**(1): 36-50.

Andalman, A. S. and M. S. Fee (2009). "A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors." Proceedings of the National Academy of Sciences **106**(30): 12518-12523.

Anderson, M. E. and R. S. Turner (1991). "Activity of neurons in cerebellar-receiving and pallidal-receiving areas of the thalamus of the behaving monkey." J Neurophysiol **66**(3): 879-893.

Aosaki, T., H. Tsubokawa, A. Ishida, K. Watanabe, A. M. Graybiel and M. Kimura (1994). "Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning." Journal of neuroscience **14**(6): 3969-3984.

Apps, R. and M. Garwicz (2005). "Anatomical and physiological foundations of cerebellar information processing." Nature Reviews Neuroscience **6**(4): 297-311.

Araya, R., K. B. Eisenthal and R. Yuste (2006). "Dendritic spines linearize the summation of excitatory potentials." Proceedings of the National Academy of Sciences **103**(49): 18799-18804.

Arends, J. and H. P. Zeigler (1991). "Organization of the cerebellum in the pigeon (Columba livia): I. Corticonuclear and corticovestibular connections." The Journal of comparative neurology **306**(2): 221-244.

Arends, J. and H. P. Zeigler (1991). "Organization of the cerebellum in the pigeon (Columba livia): II. Projections of the cerebellar nuclei." The Journal of comparative neurology **306**(2): 245-272.

Aronov, D., L. Veit, J. H. Goldberg and M. S. Fee (2011). "Two distinct modes of forebrain circuit dynamics underlie temporal patterning in the vocalizations of young songbirds." Journal of Neuroscience **31**(45): 16353-16368.

Asanuma, C., W. T. Thach and E. G. Jones (1983). "Distribution of Cerebellar Terminations and Their Relation to Other Afferent Terminations in the Ventral Lateral Thalamic Region of the Monkey." Brain Research Reviews **5**(3): 237-265.

Atoji, Y. (2011). "Immunohistochemical localization of vesicular glutamate transporter 2 (vGluT2) in the central nervous system of the pigeon (Columba livia)." Journal of Comparative Neurology **519**(14): 2887-2905.

Aumann, T. D., J. Ivanusic and M. K. Horne (1998). "Arborisation and termination of single motor thalamocortical axons in the rat." Journal of Comparative Neurology **396**(1): 121-130.

Aumann, T. D., J. A. Rawson, D. I. Finkelstein and M. K. Horne (1994). "Projections from the Lateral and Interposed Cerebellar Nuclei to the Thalamus of the Rat - a Light and Electron-Microscopic Study Using Single and Double Anterograde Labeling." Journal of Comparative Neurology **349**(2): 165-181.

Aumann, T. D., J. A. Rawson, C. Pichitpornchai and M. K. Horne (1996). "Projections from the cerebellar interposed and dorsal column nuclei to the thalamus in the rat: A double anterograde labelling study." Journal of Comparative Neurology **368**(4): 608-619.

Bastian, A. J. (2006). "Learning to predict the future: the cerebellum adapts feedforward movement control." Curr Opin Neurobiol **16**(6): 645-649.

Bauer, E. E., M. J. Coleman, T. F. Roberts, A. Roy, J. F. Prather and R. Mooney (2008). "A synaptic basis for auditory–vocal integration in the songbird." The Journal of Neuroscience **28**(6): 1509-1522.

Beecher, M. D. and E. A. Brenowitz (2005). "Functional aspects of song learning in songbirds." Trends in Ecology & Evolution **20**(3): 143-149.

Beecher, M. D. and J. M. Burt (2004). "The role of social interaction in bird song learning." Current Directions in Psychological Science **13**(6): 224-228.

Belyk, M. and S. Brown (2017). "The origins of the vocal brain in humans." Neurosci Biobehav Rev **77**: 177-193.

Berendse, H. W. and H. J. Groenewegen (1990). "Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum." Journal of Comparative Neurology **299**(2): 187-228.

Berkhoudt, H., B. G. Klein and H. P. Zeigler (1982). "Afferents to the trigeminal and facial motor nuclei in pigeon (Columba livia L.): central connections of jaw motoneurons." Journal of Comparative Neurology **209**(3): 301-312.

Bodor, A. L., K. Giber, Z. Rovo, I. Ulbert and L. Acsady (2008). "Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway." Journal of Neuroscience **28**(12): 3090-3102.

Bolhuis, J. J., K. Okanoya and C. Scharff (2010). "Twitter evolution: converging mechanisms in birdsong and human speech." Nature Reviews Neuroscience **11**(11): 747-759.

Bosch-Bouju, C., B. I. Hyland and L. C. Parr-Brownlie (2013). "Motor thalamus integration of cortical, cerebellar and basal ganglia information: implications for normal and parkinsonian conditions."

Bottjer, S., E. A. Miesner and A. P. Arnold (1984). "Forebrain lesions disrupt development but not maintenance of song in passerine birds." Science **224**: 901-904.

Bottjer, S. W. and B. Altenau (2010). "Parallel pathways for vocal learning in basal ganglia of songbirds." Nature Neuroscience **13**(2): 153-155.

Bottjer, S. W., J. D. Brady and B. Cribbs (2000). "Connections of a motor cortical region in zebra finches: relation to pathways for vocal learning." Journal of Comparative Neurology **420**(2): 244-260.

Bottjer, S. W., K. A. Halsema, S. A. Brown and E. A. Miesner (1989). "Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches." J Comp Neurol **279**(2): 312-326.

Bottjer, S. W., K. A. Halsema, S. A. Brown and E. A. Miesner (1989). "Axonal Connections of a Forebrain Nucleus Involved with Vocal Learning in Zebra Finches." Journal of Comparative Neurology **279**(2): 312-326.

Bottjer, S. W., E. A. Miesner and A. P. Arnold (1984). "Forebrain Lesions Disrupt Development but Not Maintenance of Song in Passerine Birds." Science **224**(4651): 901-903.

Bradfield, L. A., J. Bertran-Gonzalez, B. Chieng and B. W. Balleine (2013). "The Thalamostriatal Pathway and Cholinergic Control of Goal-Directed Action: Interlacing New with Existing Learning in the Striatum." Neuron **79**(1): 153-166.

Bradfield, L. A., G. Hart and B. W. Balleine (2013). "The role of the anterior, mediodorsal, and parafascicular thalamus in instrumental conditioning."

Brainard, M. S. and A. J. Doupe (2000). "Auditory feedback in learning and maintenance of vocal behaviour." Nature Reviews Neuroscience **1**(1): 31-40.

Braun, K., H. Scheich, M. Schachner and C. W. Heizmann (1985). "Distribution of parvalbumin, cytochrome oxidase activity and 14C-2-deoxyglucose uptake in the brain of the zebra finch." Cell and tissue research **240**(1): 101-115.

Braz, J. M., B. Rico and A. I. Basbaum (2002). "Transneuronal tracing of diverse CNS circuits by Cre-mediated induction of wheat germ agglutinin in transgenic mice." Proceedings of the National Academy of Sciences **99**(23): 15148-15153.

Brenowitz, E. A., D. Margoliash and K. W. Nordeen (1997). "An introduction to birdsong and the avian song system." Journal of neurobiology **33**(5): 495-500.

Brodal, P. and J. G. Bjaalie (1992). "Organization of the pontine nuclei." Neuroscience research **13**(2): 83-118.

Bukowska, D. and K. Grottel (1997). "Cells in and outside the motor trigeminal nucleus projecting to the cerebellar paramedian lobule." Neuroreport **8**(13): 2953-2956.

Busch, S., M. Selcho, K. Ito and H. Tanimoto (2009). "A map of octopaminergic neurons in the Drosophila brain." Journal of Comparative Neurology **513**(6): 643-667.

Butler, E., D. Finkelstein, M. Harvey, P. Churchward, L. Forlano and M. Horne (1996). "The relationship between monkey ventrolateral thalamic nucleus activity and kinematic parameters of wrist movement." Brain research **736**(1): 146-159.

Butler, E., M. Horne and P. Churchward (1992). "A frequency analysis of neuronal activity in monkey thalamus, motor cortex and electromyograms in wrist oscillations." The Journal of physiology **445**: 49.

Butler, E. G., D. W. Bourke and M. K. Horne (2000). "The activity of primate ventrolateral thalamic neurones during motor adaptation." Experimental brain research **133**(4).

Butler, E. G., M. K. Horne and N. J. Hawkins (1992). "The Activity of Monkey Thalamic and Motor Cortical-Neurons in a Skilled, Ballistic Movement." Journal of Physiology-London **445**: 25-48.

Carrillo, G. D. and A. J. Doupe (2004). "Is the songbird area X striatal, pallidal, or both? An anatomical study." Journal of Comparative Neurology **473**(3): 415-437.

Castelino, C. B., B. Diekamp and G. F. Ball (2007). "Noradrenergic projections to the song control nucleus area X of the medial striatum in male zebra finches (Taeniopygia guttata)." J Comp Neurol **502**(4): 544-562.

Charlesworth, J. D., E. C. Tumer, T. L. Warren and M. S. Brainard (2011). "Learning the microstructure of successful behavior." Nature neuroscience **14**(3): 373-380.

Charlesworth, J. D., T. L. Warren and M. S. Brainard (2012). "Covert skill learning in a cortical-basal ganglia circuit." Nature **486**(7402): 251-255.

Chen, C. H., R. Fremont, E. E. Arteaga-Bracho and K. Khodakhah (2014). "Short latency cerebellar modulation of the basal ganglia." Nature Neuroscience **17**(12): 1767-1775.

Chevalier, G. and J. Deniau (1982). "Inhibitory nigral influence on cerebellar evoked responses in the rat ventromedial thalamic nucleus." Experimental brain research **48**(3): 369-376.

Clarke, P. G. H. (1977). "Some Visual and Other Connections to Cerebellum of Pigeon." Journal of Comparative Neurology **174**(3): 535-552.

Clay-Ton, N. (1988). "Song tutor choice in zebra finches and Bengalese finches: the relative importance of visual and vocal cues." Behaviour **104**(3): 281-299.

Conant, R. C. and W. Ross Ashby (1970). "Every good regulator of a system must be a model of that system." International journal of systems science **1**(2): 89-97.

Consolo, S., P. Baronio, G. Guidi and G. Di Chiara (1996). "Role of the parafascicular thalamic nucleus and N-methyl-d-aspartate transmission in the D 1-dependent control of in vivo acetylcholine release in rat striatum." Neuroscience **71**(1): 157-165.

Cowie, R. and E. Douglas-Cowie (1992). Postlingually acquired deafness: speech deterioration and the wider consequences, Walter de Gruyter.

Craig, A. D. (2008). "Retrograde analyses of spinothalamic projections in the macaque monkey: Input to the ventral lateral nucleus." Journal of Comparative Neurology **508**(2): 315-328.

Darley, F. L., A. E. Aronson and J. R. Brown (1975). Motor speech disorders, Saunders.

Darshan, R., W. E. Wood, S. Peters, A. Leblois and D. Hansel (2017). "A canonical neural mechanism for behavioral variability." Nature Communications **8**: 15415.

Davis, J. F., D. L. Choi, J. D. Schurdak, M. F. Fitzgerald, D. J. Clegg, J. W. Lipton, D. P. Figlewicz and S. C. Benoit (2011). "Leptin regulates energy balance and motivation through action at distinct neural circuits." Biological psychiatry **69**(7): 668-674.

De Arcangelis, V., R. Liu, D. Soto and Y. Xiang (2009). "Differential association of phosphodiesterase 4D isoforms with β2-adrenoceptor in cardiac myocytes." Journal of Biological Chemistry **284**(49): 33824-33832.

DeLong, M. and T. Wichmann (2009). "Update on models of basal ganglia function and dysfunction." Parkinsonism & related disorders **15**: S237-S240.

Delong, M. R. (1990). "Primate Models of Movement-Disorders of Basal Ganglia Origin." Trends in Neurosciences **13**(7): 281-285.

Derégnaucourt, S., C. Poirier, A. Van der Kant, A. Van der Linden and M. Gahr (2013). "Comparisons of different methods to train a young zebra finch (Taeniopygia guttata) to learn a song." Journal of Physiology-Paris **107**(3): 210-218.

Deschenes, M., J. Bourassa, V. D. Doan and A. Parent (1996). "A single-cell study of the axonal projections arising from the posterior intralaminar thalamic nuclei in the rat." European Journal of Neuroscience **8**(2): 329-343.

Deschenes, M., J. Bourassa and A. Parent (1996). "Striatal and cortical projections of single neurons from the central lateral thalamic nucleus in the rat." Neuroscience **72**(3): 679-687.

Desmurget, M. and R. S. Turner (2008). "Testing basal ganglia motor functions through reversible inactivations in the posterior internal globus pallidus." Journal of Neurophysiology **99**(3): 1057-1076.

Desmurget, M. and R. S. Turner (2010). "Motor sequences and the basal ganglia: kinematics, not habits." Journal of Neuroscience **30**(22): 7685-7690.

Diamond, J., E. Gray and G. Yasargil (1969). "The function of dendritic spines: an analysis." The Journal of physiology **202**(2): 116P.

Dinh, T. and T. G. Bernhardt (2011). "Using superfolder green fluorescent protein for periplasmic protein localization studies." Journal of bacteriology **193**(18): 4984-4987.

Doupe, A. J. and P. K. Kuhl (1999). "Birdsong and human speech: common themes and mechanisms." Annual review of neuroscience **22**(1): 567-631.

Doupe, A. J. and P. K. Kuhl (1999). "Birdsong and human speech: Common themes and mechanisms." Annual Review of Neuroscience **22**: 567-631.

Doya, K. (1999). "What are the computations of the cerebellum, the basal ganglia and the cerebral cortex?" Neural networks **12**(7): 961-974.

Doya, K. (2000). "Complementary roles of basal ganglia and cerebellum in learning and motor control." Current opinion in neurobiology **10**(6): 732-739.

Doya, K. and T. J. Sejnowski (1998). "A computational model of birdsong learning by auditory experience and auditory feedback." Central Auditory Processing and Neural Modeling: 77-88.

Dube, L., A. D. Smith and J. P. Bolam (1988). "Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum." J Comp Neurol **267**(4): 455-471.

Dudman, J. T. and J. W. Krakauer (2016). "The basal ganglia: from motor commands to the control of vigor." Current opinion in neurobiology **37**: 158-166.

Dum, R. P. and P. L. Strick (1996). "The corticospinal system: a structural framework for the central control of movement." Comprehensive Physiology.

Ebner, T. J., A. L. Hewitt and L. S. Popa (2011). "What Features of Limb Movements are Encoded in the Discharge of Cerebellar Neurons?" Cerebellum **10**(4): 683-693.

Eckart, M. T., M. C. Huelse-Matia, R. S. McDonald and R. K.-W. Schwarting (2010). "6-Hydroxydopamine lesions in the rat neostriatum impair sequential learning in a serial reaction time task." Neurotoxicity research **17**(3): 287-298.

Edgerton, J. R. and D. Jaeger (2014). "Optogenetic activation of nigral inhibitory inputs to motor thalamus in the mouse reveals classic inhibition with little potential for rebound activation." Frontiers in Cellular Neuroscience **8**.

El-Gaby, M., Y. Zhang, K. Wolf, C. J. Schwiening, O. Paulsen and O. A. Shipton (2016). "Archaerhodopsin Selectively and Reversibly Silences Synaptic Transmission through Altered pH." Cell reports **16**(8): 2259-2268.

Elemans, C. P. (2014). "The singer and the song: The neuromechanics of avian sound production." Current opinion in neurobiology **28**: 172-178.

Ellender, T. J., J. Harwood, P. Kosillo, M. Capogna and J. P. Bolam (2013). "Heterogeneous properties of central lateral and parafascicular thalamic synapses in the striatum." Journal of Physiology-London **591**(1): 257-272.

Farries, M. A. (2001). "The oscine song system considered in the context of the avian brain: lessons learned from comparative neurobiology." Brain, behavior and evolution **58**(2): 80-100.

Farries, M. A., L. Ding and D. J. Perkel (2005). "Evidence for "direct" and "indirect" pathways through the song system basal ganglia." Journal of Comparative Neurology **484**(1): 93-104.

Farries, M. A., J. Meitzen and D. J. Perkel (2005). "Electrophysiological properties of neurons in the basal ganglia of the domestic chick: conservation and divergence in the evolution of the avian basal ganglia." Journal of neurophysiology **94**(1): 454-467.

Farries, M. A. and D. J. Perkel (2002). "A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus." Journal of Neuroscience **22**(9): 3776-3787.

Fatemi, S. H., K. A. Aldinger, P. Ashwood, M. L. Bauman, C. D. Blaha, G. J. Blatt, A. Chauhan, V. Chauhan, S. R. Dager and P. E. Dickson (2012). "Consensus paper: pathological role of the cerebellum in autism." The Cerebellum **11**(3): 777-807.

Faunes, M. and J. M. Wild (2017). "The ascending projections of the nuclei of the descending trigeminal tract (nTTD) in the zebra finch (Taeniopygia guttata)." Journal of Comparative Neurology.

Fee, M. S. (2012). "Oculomotor learning revisited: a model of reinforcement learning in the basal ganglia incorporating an efference copy of motor actions." Frontiers in Neural Circuits **6**.

Fee, M. S. and J. H. Goldberg (2011). "A hypothesis for basal ganglia-dependent reinforcement learning in the songbird." Neuroscience **198**: 152-170.

Feenders, G., M. Liedvogel, M. Rivas, M. Zapka, H. Horita, E. Hara, K. Wada, H. Mouritsen and E. D. Jarvis (2008). "Molecular mapping of movement-associated areas in the avian brain: a motor theory for vocal learning origin." PLoS One **3**(3): e1768.

Fiete, I. R., R. H. Hahnloser, M. S. Fee and H. S. Seung (2004). "Temporal sparseness of the premotor drive is important for rapid learning in a neural network model of birdsong." Journal of neurophysiology **92**(4): 2274-2282.

Finkelshtein, D., A. Werman, D. Novick, S. Barak and M. Rubinstein (2013). "LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus." Proceedings of the National Academy of Sciences **110**(18): 7306-7311.

Flourens, P. (1842). Recherches expérimentales sur les propriétés et les fonctions du système nerveux dans les animaux vertébrés, Ballière.

Fortune, E. S. and D. Margoliash (1995). "Parallel pathways and convergence onto HVc and adjacent neostriatum of adult zebra finches (Taeniopygia guttata)." Journal of Comparative Neurology **360**(3): 413-441.

Francis, B. A. and W. M. Wonham (1976). "The internal model principle of control theory." Automatica **12**(5): 457-465.

Gadagkar, V., P. A. Puzerey, R. Chen, E. Baird-Daniel, A. R. Farhang and J. H. Goldberg (2016). "Dopamine neurons encode performance error in singing birds." Science **354**(6317): 1278-1282.

Gale, S. D. and D. J. Perkel (2010). "Anatomy of a songbird basal ganglia circuit essential for vocal learning and plasticity." J Chem Neuroanat **39**(2): 124-131.

Gale, S. D., A. L. Person and D. J. Perkel (2008). "A novel basal ganglia pathway forms a loop linking a vocal learning circuit with its dopaminergic input." Journal of Comparative Neurology **508**(5): 824-839.

Galvan, A., X. Hu, Y. Smith and T. Wichmann (2016). "Effects of Optogenetic Activation of Corticothalamic Terminals in the Motor Thalamus of Awake Monkeys." Journal of Neuroscience **36**(12): 3519-3530.

Gamlin, P. D. and D. H. Cohen (1986). "A second ascending visual pathway from the optic tectum to the telencephalon in the pigeon (Columba livia)." Journal of Comparative Neurology **250**(3): 296-310.

Garcia-Calero, E. and C. Scharff (2013). "Calbindin expression in developing striatum of zebra finches and its relation to the formation of area X." Journal of Comparative Neurology **521**(2): 326-341.

Geschwind, N. (1972). Language and the brain, WH Freeman.

Ghez, C. and J. Krakauer (2000). "The organization of movement." Principles of neural science **656**: 668.

Giret, N., J. Kornfeld, S. Ganguli and R. H. Hahnloser (2014). "Evidence for a causal inverse model in an avian cortico-basal ganglia circuit." Proceedings of the National Academy of Sciences **111**(16): 6063-6068.

Goldberg, J. H., A. Adler, H. Bergman and M. S. Fee (2010). "Singing-Related Neural Activity Distinguishes Two Putative Pallidal Cell Types in the Songbird Basal Ganglia: Comparison to the Primate Internal and External Pallidal Segments." Journal of Neuroscience **30**(20): 7088-7098.

Goldberg, J. H., M. A. Farries and M. S. Fee (2012). "Integration of cortical and pallidal inputs in the basal ganglia-recipient thalamus of singing birds." Journal of neurophysiology **108**(5): 1403-1429.

Goldberg, J. H., M. A. Farries and M. S. Fee (2012). "Integration of cortical and pallidal inputs in the basal ganglia-recipient thalamus of singing birds." J Neurophysiol **108**(5): 1403-1429.

Goldberg, J. H., M. A. Farries and M. S. Fee (2013). "Basal ganglia output to the thalamus: still a paradox." Trends in neurosciences **36**(12): 695-705.

Goldberg, J. H. and M. S. Fee (2010). "Singing-Related Neural Activity Distinguishes Four Classes of Putative Striatal Neurons in the Songbird Basal Ganglia." Journal of Neurophysiology **103**(4): 2002-2014.

Goldberg, J. H. and M. S. Fee (2012). "A cortical motor nucleus drives the basal ganglia-recipient thalamus in singing birds." Nat Neurosci **15**(4): 620-627.

Goller, F., M. J. Mallinckrodt and S. D. Torti (2004). "Beak gape dynamics during song in the zebra finch." Developmental Neurobiology **59**(3): 289-303.

Goodson, J. L. (2005). "The vertebrate social behavior network: Evolutionary themes and variations." Hormones and Behavior **48**(1): 11-22.

Graham, J. (1977). "An autoradiographic study of the efferent connections of the superior colliculus in the cat." J Comp Neurol **173**(4): 629-654.

Graham, J. and N. Berman (1981). "Origins of the pretectal and tectal projections to the central lateral nucleus in the cat." Neuroscience letters **26**(3): 209-214.

Graybiel, A. M. and S. T. Grafton (2015). "The Striatum: Where Skills and Habits Meet." Cold Spring Harbor Perspectives in Biology **7**(8).

Grinevich, V., M. Brecht and P. Osten (2005). "Monosynaptic pathway from rat vibrissa motor cortex to facial motor neurons revealed by lentivirus-based axonal tracing." The Journal of neuroscience **25**(36): 8250-8258.

Gruart, A. and J. M. Delgado-Garcia (1992). "Respiration-related neurons recorded in the deep cerebellar nuclei of the alert cat." Neuroreport **3**(4): 365-368.

Hahnloser, R. H., A. A. Kozhevnikov and M. S. Fee (2002). "An ultra-sparse code underlies the generation of neural sequences in a songbird." Nature **419**(6902): 65.

Hall, Z. J., S. E. Street and S. D. Healy (2013). "The evolution of cerebellum structure correlates with nest complexity." Biology letters **9**(6): 20130687.

Hallanger, A. E., A. I. Levey, H. J. Lee, D. B. Rye and B. H. Wainer (1987). "The Origins of Cholinergic and Other Subcortical Afferents to the Thalamus in the Rat." Journal of Comparative Neurology **262**(1): 105-124.

Hampton, C. M., J. T. Sakata and M. S. Brainard (2009). "An avian basal ganglia-forebrain circuit contributes differentially to syllable versus sequence variability of adult Bengalese finch song." Journal of neurophysiology **101**(6): 3235-3245.

Hanuschkin, A., S. Ganguli and R. Hahnloser (2013). "A Hebbian learning rule gives rise to mirror neurons and links them to control theoretic inverse models." Frontiers in neural circuits **7**.

Harding, B. N. and T. P. S. Powell (1977). "Electron-Microscopic Study of Center-Median and Ventrolateral Nuclei of Thalamus in Monkey." Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **279**(966): 357-&.

Haroian, A. J., L. C. Massopust and P. A. Young (1978). "Topographical Organization of Cerebellothalamic Projections in Rat." Anatomical Record **190**(2): 414-414.

Harris, J. A., S. Wook Oh and H. Zeng (2012). "Adeno-associated viral vectors for anterograde axonal tracing with fluorescent proteins in nontransgenic and Cre driver mice." Current protocols in neuroscience: 1.20. 21-21.20. 18.

Hessler, N. A. and A. J. Doupe (1999). "Social context modulates singing-related neural activity in the songbird forebrain." Nature Neuroscience **2**(3): 209-211.

Hikosaka, O., K. Nakamura, K. Sakai and H. Nakahara (2002). "Central mechanisms of motor skill learning." Current opinion in neurobiology **12**(2): 217-222.

Hoese, W. J., J. Podos, N. C. Boetticher and S. Nowicki (2000). "Vocal tract function in birdsong production: experimental manipulation of beak movements." Journal of Experimental Biology **203**(12): 1845-1855.

Hoff, E. (2006). "How social contexts support and shape language development." Developmental review **26**(1): 55-88.

Hoffmann, L. A., V. Saravanan, A. N. Wood, L. He and S. J. Sober (2016). "Dopaminergic contributions to vocal learning." Journal of Neuroscience **36**(7): 2176-2189.

Holstege, G. (1991). "Descending motor pathways and the spinal motor system: limbic and non-limbic components." Prog Brain Res **87**: 307-421.

Horne, M. K. and E. G. Butler (1995). "The Role of the Cerebello-Thalamo-Cortical Pathway in Skilled Movement." Progress in Neurobiology **46**(2-3): 199-213.

Hoshi, E., L. Tremblay, J. Feger, P. L. Carras and P. L. Strick (2005). "The cerebellum communicates with the basal ganglia." Nature Neuroscience **8**(11): 1491-1493.

Houde, J. F. and M. I. Jordan (1998). "Sensorimotor adaptation in speech production." Science **279**(5354): 1213-1216.

Huerta, M. F., A. Frankfurter and J. K. Harting (1983). "Studies of the principal sensory and spinal trigeminal nuclei of the rat: projections to the superior colliculus, inferior olive, and cerebellum." J Comp Neurol **220**(2): 147-167.

Humphrey, D. R., R. Gold and D. J. Reed (1984). "Sizes, Laminar and Topographic Origins of Cortical Projections to the Major Divisions of the Red Nucleus in the Monkey." Journal of Comparative Neurology **225**(1): 75-94.

Ichinohe, N., H. Iwatsuki and K. Shoumura (2001). "Intrastriatal targets of projection fibers from the central lateral nucleus of the rat thalamus." Neuroscience Letters **302**(2-3): 105-108.

Ichinohe, N., F. Mori and K. Shoumura (2000). "A di-synaptic projection from the lateral cerebellar nucleus to the laterodorsal part of the striatum via the central lateral nucleus of the thalamus in the rat." Brain research **880**(1): 191-197.

Ilinsky, I. A. (1990). "Structural and connectional diversity of the primate motor thalamus: experimental light and electron microscopic studies in the rhesus monkey." Stereotactic and functional neurosurgery **54**(1-8): 114-124.

Ito, M. (1982). "Cerebellar control of the vestibulo-ocular reflex--around the flocculus hypothesis." Annual review of neuroscience **5**(1): 275-297.

Ivanusic, J. J., D. W. Bourke, Z. M. Xu, E. G. Butler and M. K. Horne (2005). "Cerebellar thalamic activity in the macaque monkey encodes the duration but not the force or velocity of wrist movement." Brain Research **1041**(2): 181-197.

Jacquin, M. F., K. Semba, R. W. Rhoades and M. D. Egger (1982). "Trigeminal Primary Afferents Project Bilaterally to Dorsal Horn and Ipsilaterally to Cerebellum, Reticular-Formation, and Cuneate, Solitary, Supratrigeminal and Vagal Nuclei." Brain Research **246**(2): 285-291.

Jahnsen, H. and R. Llinas (1984). "Electrophysiological Properties of Guinea-Pig Thalamic Neurons - an Invitro Study." Journal of Physiology-London **349**(Apr): 205-&.

Jarvis, E. D. (2004). "Learned birdsong and the neurobiology of human language." Behavioral Neurobiology of Birdsong **1016**: 749-777.

Jarvis, E. D., O. Gunturkun, L. Bruce, A. Csillag, H. Karten, W. Kuenzel, L. Medina, G. Paxinos, D. J. Perkel, T. Shimizu, G. Striedter, J. M. Wild, G. F. Ball, J. Dugas-Ford, S. E. Durand, G. E. Hough, S. Husband, L. Kubikova, D. W. Lee, C. V. Mello, A. Powers, C. Siang, T. V. Smulders, K. Wada, S. A. White, K. Yamamoto, J. Yu, A. Reiner, A. B. Butler and A. B. N. Consorti (2005). "Avian brains and a new understanding of vertebrate brain evolution." Nature Reviews Neuroscience **6**(2): 151-159.

Jiao, Y., L. Medina, C. L. Veenman, C. Toledo, L. Puelles and A. Reiner (2000). "Identification of the anterior nucleus of the ansa lenticularis in birds as the homolog of the mammalian subthalamic nucleus." J Neurosci **20**(18): 6998-7010.

Jinnah, H. and E. J. Hess (2006). "A new twist on the anatomy of dystonia The basal ganglia and the cerebellum?" Neurology **67**(10): 1740-1741.

Joel, D. and I. Weiner (1994). "The organization of the basal ganglia-thalamocortical circuits: open interconnected rather than closed segregated." Neuroscience **63**(2): 363-379.

Johnson, F., M. M. Sablan and S. W. Bottjer (1995). "Topographic organization of a forebrain pathway involved with vocal learning in zebra finches." Journal of Comparative Neurology **358**(2): 260-278.

Jones, J. A. and K. G. Munhall (2000). "Perceptual calibration of F0 production: evidence from feedback perturbation." J Acoust Soc Am **108**(3 Pt 1): 1246-1251.

Jürgens, U. (2002). "Neural pathways underlying vocal control." Neuroscience & Biobehavioral Reviews **26**(2): 235-258.

Jürgens, U. (2009). "The neural control of vocalization in mammals: a review." Journal of Voice **23**(1): 1-10.

Kao, M. H. and M. S. Brainard (2006). "Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability." Journal of neurophysiology **96**(3): 1441-1455.

Kao, M. H., A. J. Doupe and M. S. Brainard (2005). "Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song." Nature **433**(7026): 638-643.

Kappers, C. U. A., G. C. Huber and E. C. Crosby (1936). "The comparative anatomy of the nervous system of vertebrates, including man.(2 vols.)."

Karim, M. R., S. Saito and Y. Atoji (2014). "Distribution of vesicular glutamate transporter 2 in auditory and song control brain regions in the adult zebra finch (Taeniopygia guttata)." Journal of Comparative Neurology **522**(9): 2129-2151.

Karten, H. and T. E. Finger (1976). "A direct thalamo-cerebellar pathway in pigeon and catfish." Brain research **102**(2): 335-338.

Karten, H. J., A. Brzozowska-Prechtl, P. V. Lovell, D. D. Tang, C. V. Mello, H. Wang and P. P. Mitra (2013). "Digital atlas of the zebra finch (Taeniopygia guttata) brain: A high-resolution photo atlas." Journal of Comparative Neurology **521**(16): 3702-3715.

Karten, H. J. and J. L. Dubbeldam (1973). "The organization and projections of the paleostriatal complex in the pigeon (Columba livia)." Journal of Comparative Neurology **148**(1): 61-89.

Karten, H. J. and T. Shimizu (1989). "The origins of neocortex: connections and lamination as distinct events in evolution." Journal of Cognitive Neuroscience **1**(4): 291-301.

Kato, M. and M. Kimura (1992). "Effects of reversible blockade of basal ganglia on a voluntary arm movement." Journal of neurophysiology **68**(5): 1516-1534.

Kawaguchi, Y., C. J. Wilson, S. J. Augood and P. C. Emson (1995). "Striatal interneurones: chemical, physiological and morphological characterization." Trends in neurosciences **18**(12): 527-535.

Kawai, R., T. Markman, R. Poddar, R. Ko, A. L. Fantana, A. K. Dhawale, A. R. Kampff and B. P. Ölveczky (2015). "Motor cortex is required for learning but not for executing a motor skill." Neuron **86**(3): 800-812.

Kawato, M. (1999). "Internal models for motor control and trajectory planning." Current opinion in neurobiology **9**(6): 718-727.

Keen-Rhinehart, E., V. Michopoulos, D. Toufexis, E. Martin, H. Nair, K. Ressler, M. Davis, M. Owens, C. Nemeroff and M. Wilson (2009). "Continuous expression of corticotropin-releasing factor in the central nucleus of the amygdala emulates the dysregulation of the stress and reproductive axes." Molecular psychiatry **14**(1): 37-50.

Kelly, R. M. and P. L. Strick (2003). "Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate." Journal of Neuroscience **23**(23): 8432-8444.

Kemp, J. M. and T. Powell (1971). "The termination of fibres from the cerebral cortex and thalamus upon dendritic spines in the caudate nucleus: a study with the Golgi method." Philosophical Transactions of the Royal Society of London B: Biological Sciences **262**(845): 429-439.

Kemp, J. M. and T. P. S. Powell (1971). "Connexions of Striatum and Globus Pallidus - Synthesis and Speculation." Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **262**(845): 441-&.

Kennedy, P., A. Gibson and J. Houk (1986). "Functional and anatomic differentiation between parvicellular and magnocellular regions of red nucleus in the monkey." Brain research **364**(1): 124-136.

Kent, R., J. Kent, J. Rosenbek, H. Vorperian and G. Weismer (1997). "A speaking task analysis of the dysarthria in cerebellar disease." Folia Phoniatrica et Logopaedica **49**(2): 63-82.

Kent, R. D., J. F. Kent, J. R. Duffy, J. E. Thomas, G. Weismer and S. Stuntebeck (2000). "Ataxic dysarthria." Journal of Speech, Language, and Hearing Research **43**(5): 1275-1289.

Kimura, M., T. Minamimoto, N. Matsumoto and Y. Hori (2004). "Monitoring and switching of cortico-basal ganglia loop functions by the thalamo-striatal system." Neuroscience research **48**(4): 355-360.

Kitt, C. and S. Brauth (1982). "A paleostriatal-thalamic-telencephalic path in pigeons." Neuroscience **7**(11): 2735-2751.

Kobbert, C., R. Apps, I. Bechmann, J. L. Lanciego, J. Mey and S. Thanos (2000). "Current concepts in neuroanatomical tracing." Prog Neurobiol **62**(4): 327-351.

Kojima, S., M. H. Kao and A. J. Doupe (2013). "Task-related "cortical" bursting depends critically on basal ganglia input and is linked to vocal plasticity." Proceedings of the National Academy of Sciences **110**(12): 4756-4761.

Korzeniewska, E. and O. Güntürkün (1990). "Sensory properties and afferents of the N. dorsolateralis posterior thalami of the pigeon." Journal of Comparative Neurology **292**(3): 457-479.

Kotchabhakdi, N. and F. Walberg (1977). "Cerebellar afferents from neurons in motor nuclei of cranial nerves demonstrated by retrograde axonal transport of horseradish peroxidase." Brain research **137**(1): 158-163.

Kozhevnikov, A. A. and M. S. Fee (2007). "Singing-related activity of identified HVC neurons in the zebra finch." Journal of neurophysiology **97**(6): 4271-4283.

Krakauer, J. W. and P. Mazzoni (2011). "Human sensorimotor learning: adaptation, skill, and beyond." Current Opinion in Neurobiology **21**(4): 636-644.

Krout, K. E., A. D. Loewy, G. Westby and P. Redgrave (2001). "Superior colliculus projections to midline and intralaminar thalamic nuclei of the rat." Journal of Comparative Neurology **431**(2): 198-216.

Kubikova, L., K. Wada and E. D. Jarvis (2010). "Dopamine receptors in a songbird brain." Journal of Comparative Neurology **518**(6): 741-769.

Kumar, S. and S. B. Hedges (1998). "A molecular timescale for vertebrate evolution." Nature **392**(6679): 917-920.

Kuramoto, E., F. Fujiyama, K. C. Nakamura, Y. Tanaka, H. Hioki and T. Kaneko (2011). "Complementary distribution of glutamatergic cerebellar and GABAergic basal ganglia afferents to the rat motor thalamic nuclei." European Journal of Neuroscience **33**(1): 95-109.

Kuramoto, E., T. Furuta, K. C. Nakamura, T. Unzai, H. Hioki and T. Kaneko (2009). "Two types of thalamocortical projections from the motor thalamic nuclei of the rat: a single neuron-tracing study using viral vectors." Cerebral Cortex **19**(9): 2065-2077.

Kuypers, H. (1981). "Anatomy of the descending pathways." Comprehensive Physiology.

Lacey, C. J., J. P. Bolam and P. J. Magill (2007). "Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections." Journal of Neuroscience **27**(16): 4374-4384.

Lapper, S. R. and J. P. Bolam (1992). "Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat." Neuroscience **51**(3): 533-545.

Lavoie, B. and A. Parent (1991). "Serotoninergic Innervation of the Thalamus in the Primate - an Immunohistochemical Study." Journal of Comparative Neurology **312**(1): 1-18.

Lavoie, B. and A. Parent (1994). "Pedunculopontine Nucleus in the Squirrel-Monkey - Projections to the Basal Ganglia as Revealed by Anterograde Tract-Tracing Methods." Journal of Comparative Neurology **344**(2): 210-231.

Leblois, A., Á. L. Bodor, A. L. Person and D. J. Perkel (2009). "Millisecond timescale disinhibition mediates fast information transmission through an avian basal ganglia loop." Journal of Neuroscience **29**(49): 15420-15433.

Lewis, J. W., S. M. Ryan, A. P. Arnold and L. L. Butcher (1981). "Evidence for a catecholaminergic projection to area X in the zebra finch." Journal of Comparative Neurology **196**(2): 347-354.

Li, J., X. Zhou, L. Huang, X. Fu, J. Liu, X. Zhang, Y. Sun and M. Zuo (2013). "Alteration of CaBP Expression Pattern in the Nucleus Magnocellularis following Unilateral Cochlear Ablation in Adult Zebra Finches." PloS one **8**(11): e79297.

Lindberg, D., P. Chen and C. Li (2013). "Conditional viral tracing reveals that steroidogenic factor 1-positive neurons of the dorsomedial subdivision of the ventromedial hypothalamus project to autonomic centers of the hypothalamus and hindbrain." Journal of Comparative Neurology **521**(14): 3167-3190.

Liu, Y., J. Luo, M. A. Carlsson and D. R. Nässel (2015). "Serotonin and insulin-like peptides modulate leucokinin-producing neurons that affect feeding and water homeostasis in Drosophila." Journal of Comparative Neurology **523**(12): 1840-1863.

Lu, L. Y., Y. Cao, K. Tokita, D. H. Heck and J. D. Boughter (2013). "Medial cerebellar nuclear projections and activity patterns link cerebellar output to orofacial and respiratory behavior." Frontiers in Neural Circuits **7**.

Luo, M., L. Ding and D. J. Perkel (2001). "An avian basal ganglia pathway essential for vocal learning forms a closed topographic loop." Journal of Neuroscience **21**(17): 6836-6845.

Luo, M. and D. J. Perkel (1999). "A GABAergic, strongly inhibitory projection to a thalamic nucleus in the zebra finch song system." Journal of Neuroscience **19**(15): 6700-6711.

Luo, M. and D. J. Perkel (1999). "Long-range GABAergic projection in a circuit essential for vocal learning." The Journal of comparative neurology **403**(1): 68-84.

Lutherer, L. O., J. L. Williams and S. J. Everse (1989). "Neurons of the Rostral Fastigial Nucleus Are Responsive to Cardiovascular and Respiratory Challenges." Journal of the Autonomic Nervous System **27**(2): 101-112.

Ma, W., M. Peschanski and H. J. Ralston (1987). "Fine-Structure of the Spinothalamic Projections to the Central Lateral Nucleus of the Rat Thalamus." Brain Research **414**(1): 187-191.

Mahn, M., M. Prigge, S. Ron, R. Levy and O. Yizhar (2016). "Biophysical constraints of optogenetic inhibition at presynaptic terminals." Nature neuroscience **19**(4): 554-556.

Mandelblat-Cerf, Y. and M. S. Fee (2014). "An automated procedure for evaluating song imitation." PloS one **9**(5): e96484.

Mandelblat-Cerf, Y., L. Las, N. Denisenko and M. S. Fee (2014). "A role for descending auditory cortical projections in songbird vocal learning." Elife **3**: e02152.

Manto, M., J. M. Bower, A. B. Conforto, J. M. Delgado-Garcia, S. N. F. da Guarda, M. Gerwig, C. Habas, N. Hagura, R. B. Ivry, P. Marien, M. Molinari, E. Naito, D. A. Nowak, N. O. Ben Taib, D. Pelisson, C. D. Tesche, C. Tilikete and D. Timmann (2012). "Consensus Paper: Roles of the Cerebellum in Motor Control-The Diversity of Ideas on Cerebellar Involvement in Movement." Cerebellum **11**(2): 457-487.

Marien, P., H. Ackermann, M. Adamaszek, C. H. S. Barwood, A. Beaton, J. Desmond, E. De Witte, A. J. Fawcett, I. Hertrich, M. Kuper, M. Leggio, C. Marvel, M. Molinari, B. E. Murdoch, R. I. Nicolson, J. D. Schmahmann, C. J. Stoodley, M. Thurling, D. Timmann, E. Wouters and W. Ziegler (2014). "Consensus Paper: Language and the Cerebellum: an Ongoing Enigma." Cerebellum **13**(3): 386-410.

Marler, P. (1970). "Birdsong and speech development: Could there be parallels? There may be basic rules governing vocal learning to which many species conform, including man." American scientist **58**(6): 669-673.

Marler, P. (1970). "A Comparative Approach to Vocal Learning - Song Development in White-Crowned Sparrows." Journal of Comparative and Physiological Psychology **71**(2): 1-&.

Marler, P. and M. Tamura (1964). "Culturally Transmitted Patterns of Vocal Behavior in Sparrows." Science **146**(3650): 1483-1486.

Martin, T. A., J. G. Keating, H. P. Goodkin, A. J. Bastian and W. T. Thach (1996). "Throwing while looking through prisms. I. Focal olivocerebellar lesions impair adaptation." Brain **119 (Pt 4)**: 1183-1198.

Martin, T. A., J. G. Keating, H. P. Goodkin, A. J. Bastian and W. T. Thach (1996). "Throwing while looking through prisms. II. Specificity and storage of multiple gaze-throw calibrations." Brain **119 (Pt 4)**: 1199-1211.

Matsumoto, N., T. Minamimoto, A. M. Graybiel and M. Kimura (2001). "Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events." Journal of Neurophysiology **85**(2): 960-976.

Mauk, M., J. Medina, W. Nores and T. Ohyama (2000). "Cerebellar function: coordination, learning or timing?" Current Biology **10**(14): R522-R525.

Mazzoni, P., A. Hristova and J. W. Krakauer (2007). "Why don't we move faster? Parkinson's disease, movement vigor, and implicit motivation." Journal of neuroscience **27**(27): 7105-7116.

McDougle, S. D., M. J. Boggess, M. J. Crossley, D. Parvin, R. B. Ivry and J. A. Taylor (2016). "Credit assignment in movement-dependent reinforcement learning." Proceedings of the National Academy of Sciences: 201523669.

McFarland, N. R. and S. N. Haber (2001). "Organization of thalamostriatal terminals from the ventral motor nuclei in the macaque." Journal of Comparative Neurology **429**(2): 321-336.

McHaffie, J. G., T. R. Stanford, B. E. Stein, W. Coizet and P. Redgrave (2005). "Subcortical loops through the basal ganglia." Trends in Neurosciences **28**(8): 401-407.

McKenna, J. T., C. Yang, S. Franciosi, S. Winston, K. K. Abarr, M. S. Rigby, Y. Yanagawa, R. W. McCarley and R. E. Brown (2013). "Distribution and intrinsic membrane properties of basal forebrain GABAergic and parvalbumin neurons in the mouse." Journal of Comparative Neurology **521**(6): 1225-1250.

Medina, J. F., K. S. Garcia, W. L. Nores, N. M. Taylor and M. D. Mauk (2000). "Timing mechanisms in the cerebellum: testing predictions of a large-scale computer simulation." Journal of Neuroscience **20**(14): 5516-5525.

Medina, L. and A. Reiner (1994). "Distribution of Choline-Acetyltransferase Immunoreactivity in the Pigeon Brain." Journal of Comparative Neurology **342**(4): 497-537.

Medina, L. and A. Reiner (1997). "The efferent projections of the dorsal and ventral pallidal parts of the pigeon basal ganglia, studied with biotinylated dextran amine." Neuroscience **81**(3): 773-802.

Medina, L., C. L. Veenman and A. Reiner (1997). "Evidence for a possible avian dorsal thalamic region comparable to the mammalian ventral anterior, ventral lateral, and oral ventroposterolateral nuclei." The Journal of comparative neurology **384**(1): 86-108.

Middleton, F. A. and P. L. Strick (2000). "Basal ganglia and cerebellar loops: motor and cognitive circuits." Brain research reviews **31**(2): 236-250.

Minamimoto, T., Y. Hori and M. Kimura (2009). "Roles of the thalamic CM–PF complex—basal ganglia circuit in externally driven rebias of action." Brain research bulletin **78**(2): 75-79.

Mink, J. W. (1996). "The basal ganglia: focused selection and inhibition of competing motor programs." Progress in neurobiology **50**(4): 381-425.

Miyachi, S., O. Hikosaka, K. Miyashita, Z. Kárádi and M. K. Rand (1997). "Differential roles of monkey striatum in learning of sequential hand movement." Experimental brain research **115**(1): 1-5.

Moruzzi, G. and H. W. Magoun (1949). "Brain stem reticular formation and activation of the EEG." Electroencephalography and clinical neurophysiology **1**(1): 455-473.

Moss, J. and J. P. Bolam (2008). "A Dopaminergic Axon Lattice in the Striatum and Its Relationship with Cortical and Thalamic Terminals." Journal of Neuroscience **28**(44): 11221-11230.

Moussa, R., B. Poucet, M. Amalric and F. Sargolini (2011). "Contributions of dorsal striatal subregions to spatial alternation behavior." Learning & Memory **18**(7): 444-451.

Mundell, N. A., K. T. Beier, Y. A. Pan, S. W. Lapan, D. Göz Aytürk, V. K. Berezovskii, A. R. Wark, E. Drokhlyansky, J. Bielecki, R. T. Born, A. F. Schier and C. L. Cepko (2015). "Vesicular stomatitis virus enables gene transfer and transsynaptic tracing in a wide range of organisms." Journal of Comparative Neurology **523**(11): 1639-1663.

Mushiake, H. and P. L. Strick (1993). "Preferential Activity of Dentate Neurons during Limb Movements Guided by Vision." Journal of Neurophysiology **70**(6): 2660-2664.

Mushiake, H. and P. L. Strick (1995). "Pallidal neuron activity during sequential arm movements." Journal of Neurophysiology **74**(6): 2754-2758.

Nakamura, K. C., A. Sharott and P. J. Magill (2014). "Temporal coupling with cortex distinguishes spontaneous neuronal activities in identified basal ganglia-recipient and cerebellar-recipient zones of the motor thalamus." Cereb Cortex **24**(1): 81-97.

Nambu, A. (2004). "A new dynamic model of the cortico-basal ganglia loop." Progress in brain research **143**: 461-466.

Nambu, A. (2008). "Seven problems on the basal ganglia." Current Opinion in Neurobiology **18**(6): 595-604.

Nambu, A., S. Yoshida and K. Jinnai (1991). "Movement-related activity of thalamic neurons with input from the globus pallidus and projection to the motor cortex in the monkey." Experimental brain research **84**(2): 279-284.

Nanda, B., A. Galvan, Y. Smith and T. Wichmann (2009). "Effects of stimulation of the centromedian nucleus of the thalamus on the activity of striatal cells in awake rhesus monkeys." European Journal of Neuroscience **29**(3): 588-598.

Nelson, B. S., G. J. Beckers and R. A. Suthers (2005). "Vocal tract filtering and sound radiation in a songbird." Journal of Experimental Biology **208**(2): 297-308.

Nelson, D. A., P. Marler and A. Palleroni (1995). "A Comparative Approach to Vocal Learning - Intraspecific Variation in the Learning-Process." Animal Behaviour **50**: 83-97.

Newman, D. and C. Ginsberg (1993). "Brainstem reticular nuclei that project to the thalamus in rats: a retrograde tracer study." Brain, behavior and evolution **44**(1): 1-39.

Newman, S. W. (1999). "The medial extended amygdala in male reproductive behavior - A node in the mammalian social behavior network." Advancing from the Ventral Striatum to the Extended Amygdala **877**: 242-257.

Neychev, V. K., X. Fan, V. Mitev, E. J. Hess and H. Jinnah (2008). "The basal ganglia and cerebellum interact in the expression of dystonic movement." Brain **131**(9): 2499-2509.

Nimchinsky, E. A., B. L. Sabatini and K. Svoboda (2002). "Structure and function of dendritic spines." Annual review of physiology **64**(1): 313-353.

Nixdorf-Bergweiler, B. E., M. B. Lips and U. Heinemann (1995). "Electrophysiological and morphological evidence for a new projection of LMAN-neurones towards area X." Neuroreport **6**(13): 1729-1732.

Nordeen, K. W. and E. J. Nordeen (1993). "Long-term maintenance of song in adult zebra finches is not affected by lesions of a forebrain region involved in song learning." Behavioral and neural biology **59**(1): 79-82.

Nottebohm, F., J. A. Paton and D. B. Kelley (1982). "Connections of vocal control nuclei in the canary telencephalon." Journal of Comparative Neurology **207**(4): 344-357.

Nottebohm, F., T. M. Stokes and C. M. Leonard (1976). "Central Control of Song in Canary, Serinus-Canarius." Journal of Comparative Neurology **165**(4): 457-486.

Nottebohm, F., T. M. Stokes and C. M. Leonard (1976). "Central control of song in the canary, Serinus canarius." Journal of Comparative Neurology **165**(4): 457-486.

Nottebohm, F., T. M. Stokes and C. M. Leonard (1976). "Central control of song in the canary, Serinus canarius." J Comp Neurol **165**(4): 457-486.

Nowicki, S. (1987). "Vocal tract resonances in oscine bird sound production: evidence from birdsongs in a helium atmosphere." Nature **325**(6099): 53-55.

Okanoya, K. and A. Yamaguchi (1997). "Adult Bengalese finches (Lonchura striata var. domestica) require real-time auditory feedback to produce normal song syntax." J Neurobiol **33**(4): 343-356.

Optican, L. M. and D. A. Robinson (1980). "Cerebellar-dependent adaptive control of primate saccadic system." Journal of Neurophysiology **44**(6): 1058-1076.

Pakan, J. M. and D. R. Wylie (2006). "Two optic flow pathways from the pretectal nucleus lentiformis mesencephali to the cerebellum in pigeons (Columba livia)." J Comp Neurol **499**(5): 732-744.

Pare, D., Y. Smith, A. Parent and M. Steriade (1988). "Projections of brainstem core cholinergic and non-cholinergic neurons of cat to intralaminar and reticular thalamic nuclei." Neuroscience **25**(1): 69-86.

Parent, M. and A. Parent (2005). "Single-axon tracing and three-dimensional reconstruction of centre median-parafascicular thalamic neurons in primates." Journal of Comparative Neurology **481**(1): 127-144.

Parker, P. R., A. L. Lalive and A. C. Kreitzer (2016). "Pathway-specific remodeling of thalamostriatal synapses in parkinsonian mice." Neuron **89**(4): 734-740.

Penney, J. B. and A. B. Young (1983). "Speculations on the Functional-Anatomy of Basal Ganglia Disorders." Annual Review of Neuroscience **6**: 73-94.

Percheron, G., C. Francois, B. Talbi, J. Yelnik and G. Fenelon (1996). "The primate motor thalamus." Brain Research Reviews **22**(2): 93-181.

Perrett, S. P., B. P. Ruiz and M. D. Mauk (1993). "Cerebellar cortex lesions disrupt learning-dependent timing of conditioned eyelid responses." Journal of Neuroscience **13**: 1708-1708.

Person, A. L., S. D. Gale, M. A. Farries and D. J. Perkel (2008). "Organization of the songbird basal ganglia, including area X." J Comp Neurol **508**(5): 840-866.

Person, A. L., S. D. Gale, M. A. Farries and D. J. Perkel (2008). "Organization of the songbird basal ganglia, including Area X." Journal of Comparative Neurology **508**(5): 840-866.

Person, A. L. and D. J. Perkel (2005). "Unitary IPSPs drive precise thalamic spiking in a circuit required for learning." Neuron **46**(1): 129-140.

Pinault, D. (1996). "A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin." Journal of neuroscience methods **65**(2): 113-136.

Podos, J., J. A. Southall and M. R. Rossi-Santos (2004). "Vocal mechanics in Darwin's finches: correlation of beak gape and song frequency." Journal of experimental biology **207**(4): 607-619.

Raju, D. V., D. J. Shah, T. M. Wright, R. A. Hall and Y. Smith (2006). "Differential synaptology of vGluT2-containing thalamostriatal afferents between the patch and matrix compartments in rats." Journal of Comparative Neurology **499**(2): 231-243.

Redondo, R. L., J. Kim, A. L. Arons, S. Ramirez, X. Liu and S. Tonegawa (2014). "Bidirectional switch of the valence associated with a hippocampal contextual memory engram." Nature **513**(7518): 426-430.

Reiner, A., N. C. Brecha and H. J. Karten (1982). "Basal ganglia pathways to the tectum: the afferent and efferent connections of the lateral spiriform nucleus of pigeon." Journal of Comparative Neurology **208**(1): 16-36.

Reiner, A., A. V. Laverghetta, C. A. Meade, S. L. Cuthbertson and S. W. Bottjer (2004). "An immunohistochemical and pathway tracing study of the striatopallidal organization of area X in the male zebra finch." Journal of Comparative Neurology **469**(2): 239-261.

Reiner, A., L. Medina and C. L. Veenman (1998). "Structural and functional evolution of the basal ganglia in vertebrates." Brain Res Brain Res Rev **28**(3): 235-285.

Reiner, A., D. J. Perkel, L. L. Bruce, A. B. Butler, A. Csillag, W. Kuenzel, L. Medina, G. Paxinos, T. Shimizu and G. Striedter (2004). "Revised nomenclature for avian telencephalon and some related brainstem nuclei." Journal of Comparative Neurology **473**(3): 377-414.

Reiner, A., D. J. Perkel, L. L. Bruce, A. B. Butler, A. Csillag, W. Kuenzel, L. Medina, G. Paxinos, T. Shimizu, G. Striedter, M. Wild, G. F. Ball, S. Durand, O. Gunturkun, D. W. Lee, C. V. Mello, A. Powers, S. A. White, G. Hough, L. Kubikova, T. V. Smulders, K. Wada, J. Dugas-Ford, S. Husband, K. Yamamoto, J. Yu, C. Siang and E. D. Jarvis (2004). "Revised nomenclature for avian telencephalon and some related brainstem nuclei." Journal of Comparative Neurology **473**(3): 377-414.

Reiner, A., D. J. Perkel, C. V. Mello and E. D. Jarvis (2004). "Songbirds and the revised avian brain nomenclature." Behavioral Neurobiology of Birdsong **1016**: 77-108.

Reiner, A., C. L. Veenman, L. Medina, Y. Jiao, N. Del Mar and M. G. Honig (2000). "Pathway tracing using biotinylated dextran amines." J Neurosci Methods **103**(1): 23-37.

Riede, T., R. A. Suthers, N. H. Fletcher and W. E. Blevins (2006). "Songbirds tune their vocal tract to the fundamental frequency of their song." Proceedings of the National Academy of Sciences **103**(14): 5543-5548.

Rinvik, E. and I. Grofova (1974). "Cerebellar Projections to Nuclei Ventralis Lateralis and Ventralis Anterior Thalami - Experimental Electron Microscopical and Light Microscopical Studies in Cat." Anatomy and Embryology **146**(1): 95-111.

Roberts, T. F., M. E. Klein, M. F. Kubke, J. M. Wild and R. Mooney (2008). "Telencephalic neurons monosynaptically link brainstem and forebrain premotor networks necessary for song." The Journal of Neuroscience **28**(13): 3479-3489.

Rochefort, C., X. He, S. Scotto-Lomassese and C. Scharff (2007). "Recruitment of FoxP2-expressing neurons to area X varies during song development." Developmental Neurobiology **67**(6): 809-817.

Royce, G. J. (1983). "Single thalamic neurons which project to both the rostral cortex and caudate nucleus studied with the fluorescent double labeling method." Experimental neurology **79**(3): 773-784.

Sadananda, M. (2004). "Acetylcholinesterase in central vocal control nuclei of the zebra finch (Taeniopygia guttata)." Journal of biosciences **29**(2): 189-200.

Sadikot, A. F., A. Parent, Y. Smith and J. P. Bolam (1992). "Efferent Connections of the Centromedian and Parafascicular Thalamic Nuclei in the Squirrel-Monkey - a Light and Electron-Microscopic Study of the Thalamostriatal Projection in Relation to Striatal Heterogeneity." Journal of Comparative Neurology **320**(2): 228-242.

Saigal, R., A. Karamanlidis, J. Voogd, H. Michaloudi and O. Mangana (1980). "Cerebellar afferents from motor nuclei of cranial nerves, the nucleus of the solitary tract, and nuclei coeruleus and parabrachialis in sheep, demonstrated with retrograde transport of horseradish peroxidase." Brain research **197**(1): 200-206.

Schnyder, H., H. Reisine, K. Hepp and V. Henn (1985). "Frontal eye field projection to the paramedian pontine reticular formation traced with wheat germ agglutinin in the monkey." Brain research **329**(1): 151-160.

Schoch, B., A. Dimitrova, E. Gizewski and D. Timmann (2006). "Functional localization in the human cerebellum based on voxelwise statistical analysis: a study of 90 patients." Neuroimage **30**(1): 36-51.

Schultz, W., P. Dayan and P. R. Montague (1997). "A neural substrate of prediction and reward." Science **275**(5306): 1593-1599.

Schwarz, C. and Y. Schmitz (1997). "Projection from the cerebellar lateral nucleus to precerebellar nuclei in the mossy fiber pathway is glutamatergic: a study combining anterograde tracing with immunogold labeling in the rat." Journal of Comparative Neurology **381**(3): 320-334.

Schwarz, L. A., K. Miyamichi, X. J. Gao, K. T. Beier, B. Weissbourd, K. E. DeLoach, J. Ren, S. Ibanes, R. C. Malenka and E. J. Kremer (2015). "Viral-genetic tracing of the input-output organization of a central noradrenaline circuit." Nature.

Sedgwick, E. and T. Williams (1967). "Responses of single units in the inferior olive to stimulation of the limb nerves, peripheral skin receptors, cerebellum, caudate nucleus and motor cortex." The Journal of physiology **189**(2): 261.

Shadmehr, R. and J. W. Krakauer (2008). "A computational neuroanatomy for motor control." Experimental Brain Research **185**(3): 359-381.

Sherman, S. M. and R. W. Guillery (1998). "On the actions that one nerve cell can have on another: Distinguishing "drivers" from "modulators"." Proceedings of the National Academy of Sciences of the United States of America **95**(12): 7121-7126.

Shinoda, Y., T. Futami and M. Kano (1985). "Synaptic organization of the cerebello-thalamo-cerebral pathway in the cat. II. Input-output organization of single thalamocortical neurons in the ventrolateral thalamus." Neuroscience research **2**(3): 157-180.

Shinoda, Y., T. Futami, H. Mitoma and J. Yokota (1988). "Morphology of single neurones in the cerebello-rubrospinal system." Behav Brain Res **28**(1-2): 59-64.

Shmuelof, L. and J. W. Krakauer (2011). "Are we ready for a natural history of motor learning?" Neuron **72**(3): 469-476.

Sidibe, M. and Y. Smith (1999). "Thalamic inputs to striatal interneurons in monkeys: synaptic organization and co-localization of calcium binding proteins." Neuroscience **89**(4): 1189-1208.

Simonyan, K. and U. Jürgens (2003). "Efferent subcortical projections of the laryngeal motorcortex in the rhesus monkey." Brain research **974**(1): 43-59.

Simonyan, K. and U. Jürgens (2005). "Afferent subcortical connections into the motor cortical larynx area in the rhesus monkey." Neuroscience **130**(1): 119-131.

Smith, A. D. and J. P. Bolam (1990). "The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones." Trends in neurosciences **13**(7): 259-265.

Smith, Y., B. D. Bennett, J. P. Bolam, A. Parent and A. F. Sadikot (1994). "Synaptic Relationships between Dopaminergic Afferents and Cortical or Thalamic Input in the Sensorimotor Territory of the Striatum in Monkey." Journal of Comparative Neurology **344**(1): 1-19.

Smith, Y., A. Galvan, T. J. Ellender, N. Doig, R. M. Villalba, I. Huerta-Ocampo, T. Wichmann and J. P. Bolam (2014). "The thalamostriatal system in normal and diseased states."

Smith, Y. and A. Parent (1986). "Differential Connections of Caudate-Nucleus and Putamen in the Squirrel-Monkey (Saimiri-Sciureus)." Neuroscience **18**(2): 347-&.

Snider, R. S. and A. Maiti (1976). "Cerebellar contributions to the Papez circuit." Journal of neuroscience research **2**(2): 133-146.

Sober, S. J. and M. S. Brainard (2009). "Adult birdsong is actively maintained by error correction." Nat Neurosci **12**(7): 927-931.

Sober, S. J. and M. S. Brainard (2009). "Adult birdsong is actively maintained by error correction." Nature Neuroscience **12**(7): 927-U144.

Sober, S. J. and M. S. Brainard (2012). "Vocal learning is constrained by the statistics of sensorimotor experience." Proc Natl Acad Sci U S A **109**(51): 21099-21103.

Sohrabji, F., E. J. Nordeen and K. W. Nordeen (1990). "Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch." Behavioral and neural biology **53**(1): 51-63.

Sommer, M. A. (2003). "The role of the thalamus in motor control." Curr Opin Neurobiol **13**(6): 663-670.

Sommer, M. A. and R. H. Wurtz (2002). "A pathway in primate brain for internal monitoring of movements." Science **296**(5572): 1480-1482.

Spence, R. D., Y. Zhen, S. White, B. A. Schlinger and L. B. Day (2009). "Recovery of motor and cognitive function after cerebellar lesions in a songbird–role of estrogens." European Journal of Neuroscience **29**(6): 1225-1234.

Spencer, K. A. and D. L. Slocomb (2007). "The neural basis of ataxic dysarthria." Cerebellum **6**(1): 58-65.

Sreenivasan, V., K. Karmakar, F. M. Rijli and C. C. Petersen (2015). "Parallel pathways from motor and somatosensory cortex for controlling whisker movements in mice." European Journal of Neuroscience **41**(3): 354-367.

Stepanek, L. and A. J. Doupe (2010). "Activity in a cortical-basal ganglia circuit for song is required for social context-dependent vocal variability." Journal of neurophysiology **104**(5): 2474-2486.

Stepniewska, I., S. T. Sakai, H. X. Qi and J. H. Kaas (2003). "Somatosensory input to the ventrolateral thalamic region in the macaque monkey: A potential substrate for parkinsonian tremor." Journal of Comparative Neurology **455**(3): 378-395.

Steriade, M., D. Pare, A. Parent and Y. Smith (1988). "Projections of cholinergic and non-cholinergic neurons of the brainstem core to relay and associational thalamic nuclei in the cat and macaque monkey." Neuroscience **25**(1): 47-67.

Streng, M. L., L. S. Popa and T. J. Ebner (2017). "Climbing fibers predict movement kinematics and performance errors." Journal of Neurophysiology **118**(3): 1888-1902.

Sugimoto, T., N. Mizuno and K. Itoh (1981). "An autoradiographic study on the terminal distribution of cerebellothalamic fibers in the cat." Brain Res **215**(1-2): 29-47.

Sutton, R. S. and A. G. Barto (1998). Reinforcement learning: An introduction, MIT press Cambridge.

Taylor, J. A. and R. B. Ivry (2014). "Cerebellar and prefrontal cortex contributions to adaptation, strategies, and reinforcement learning." Progress in brain research **210**: 217.

Taylor, J. A., J. W. Krakauer and R. B. Ivry (2014). "Explicit and implicit contributions to learning in a sensorimotor adaptation task." Journal of Neuroscience **34**(8): 3023-3032.

Tchernichovski, O., P. P. Mitra, T. Lints and F. Nottebohm (2001). "Dynamics of the vocal imitation process: How a zebra finch learns its song." Science **291**(5513): 2564-2569.

Tchernichovski, O., F. Nottebohm, C. E. Ho, B. Pesaran and P. P. Mitra (2000). "A procedure for an automated measurement of song similarity." Animal behaviour **59**(6): 1167-1176.

Thach, W. T. (2014). "Does the Cerebellum Initiate Movement?" Cerebellum **13**(1): 139-150.

Thoms, G. and U. Jürgens (1987). "Common input of the cranial motor nuclei involved in phonation in squirrel monkey." Experimental neurology **95**(1): 85-99.

Tomasello, M. (2009). Constructing a language, Harvard university press.

Tracey, D. J., C. Asanuma, E. G. Jones and R. Porter (1980). "Thalamic Relay to Motor Cortex - Afferent Pathways from Brain-Stem, Cerebellum, and Spinal-Cord in Monkeys." Journal of Neurophysiology **44**(3): 532-554.

Tremblay, S., D. M. Shiller and D. J. Ostry (2003). "Somatosensory basis of speech production." Nature **423**(6942): 866-869.

Troyer, T. W. and A. J. Doupe (2000). "An associational model of birdsong sensorimotor learning I. Efference copy and the learning of song syllables." Journal of Neurophysiology **84**(3): 1204-1223.

Troyer, T. W. and A. J. Doupe (2000). "An associational model of birdsong sensorimotor learning II. Temporal hierarchies and the learning of song sequence." Journal of Neurophysiology **84**(3): 1224-1239.

Tumer, E. C. and M. S. Brainard (2007). "Performance variability enables adaptive plasticity of 'crystallized' adult birdsong." Nature **450**(7173): 1240-1244.

Turner, R. S. and M. Desmurget (2010). "Basal ganglia contributions to motor control: a vigorous tutor." Current Opinion in Neurobiology **20**(6): 704-716.

Ueki, A. (1983). "The mode of nigro-thalamic transmission investigated with intracellular recording in the cat." Experimental brain research **49**(1): 116-124.

Uno, M., M. Yoshida and I. Hirota (1970). "The mode of cerebello-thalamic relay transmission investigated with intracellular recording from cells of the ventrolateral nucleus of cat's thalamus." Experimental brain research **10**(2): 121-139.

Urban, P. P., J. Marx, S. Hunsche, J. Gawehn, G. Vucurevic, S. Wicht, C. Massinger, P. Stoeter and H. C. Hopf (2003). "Cerebellar speech representation: lesion topography in dysarthria as

derived from cerebellar ischemia and functional magnetic resonance imaging." Archives of Neurology **60**(7): 965-972.

Van der Werf, Y. D., M. P. Witter and H. J. Groenewegen (2002). "The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness." Brain research reviews **39**(2): 107-140.

Van Donkelaar, P., J. Stein, R. Passingham and R. Miall (1999). "Neuronal activity in the primate motor thalamus during visually triggered and internally generated limb movements." Journal of Neurophysiology **82**(2): 934-945.

Van Donkelaar, P., J. Stein, R. Passingham and R. Miall (2000). "Temporary inactivation in the primate motor thalamus during visually triggered and internally generated limb movements." Journal of Neurophysiology **83**(5): 2780-2790.

VanderHorst, V., E. Terasawa and H. Ralston (2001). "Monosynaptic projections from the nucleus retroambiguus region to laryngeal motoneurons in the rhesus monkey." Neuroscience **107**(1): 117-125.

Vanderhorst, V. G., E. Terasawa, H. J. Ralston and G. Holstege (2000). "Monosynaptic projections from the lateral periaqueductal gray to the nucleus retroambiguus in the rhesus monkey: implications for vocalization and reproductive behavior." Journal of Comparative Neurology **424**(2): 251-268.

Vates, G. E. and F. Nottebohm (1995). "Feedback circuitry within a song-learning pathway." Proceedings of the National Academy of Sciences **92**(11): 5139-5143.

Vates, G. E., D. S. Vicario and F. Nottebohm (1997). "Reafferent thalamo-"cortical" loops in the song system of oscine songbirds." Journal of Comparative Neurology **380**(2): 275-290.

Veenman, C. L., E. J. Karle, K. D. Anderson and A. Reiner (1995). "Thalamostriatal projection neurons in birds utilize LANT6 and neurotensin: a light and electron microscopic double-labeling study." J Chem Neuroanat **9**(1): 1-16.

Veenman, C. L., L. Medina and A. Reiner (1997). "Avian homologues of mammalian intralaminar, mediodorsal and midline thalamic nuclei: Immunohistochemical and hodological evidence." Brain Behavior and Evolution **49**(2): 78-98.

Veenman, C. L., L. Medina and A. Reiner (1997). "Avian homologues of mammalian intralaminar, mediodorsal and midline thalamic nuclei: immunohistochemical and hodological evidence." Brain Behav Evol **49**(2): 78-98.

Vertes, R. P. (1991). "A Pha-L Analysis of Ascending Projections of the Dorsal Raphe Nucleus in the Rat." Journal of Comparative Neurology **313**(4): 643-668.

Vertes, R. P., W. J. Fortin and A. M. Crane (1999). "Projections of the median raphe nucleus in the rat." Journal of Comparative Neurology **407**(4): 555-582.

Vertes, R. P. and G. F. Martin (1988). "Autoradiographic Analysis of Ascending Projections from the Pontine and Mesencephalic Reticular-Formation and the Median Raphe Nucleus in the Rat." Journal of Comparative Neurology **275**(4): 511-541.

Vicario, D. S. (1991). "Organization of the zebra finch song control system: functional organization of outputs from nucleus robustus archistriatalis." Journal of Comparative Neurology **309**(4): 486-494.

Vogt, C. (1909). La myéloarchitecture du thalamus du cercopithèque.

Vujovic, N., J. J. Gooley, T. C. Jhou and C. B. Saper (2015). "Projections from the subparaventricular zone define four channels of output from the circadian timing system." Journal of Comparative Neurology **523**(18): 2714-2737.

Walker, A. E. (1938). "The primate thalamus."

Wang, S. S.-H., A. D. Kloth and A. Badura (2014). "The cerebellum, sensitive periods, and autism." Neuron **83**(3): 518-532.

Wild, J. (1987). "Thalamic projections to the paleostriatum and neostriatum in the pigeon (Columba livia)." Neuroscience **20**(1): 305-327.

Wild, J. and N. Krützfeldt (2012). "Trigeminal and telencephalic projections to jaw and other upper vocal tract premotor neurons in songbirds: sensorimotor circuitry for beak movements during singing." Journal of Comparative Neurology **520**(3): 590-605.

Wild, J., M. Kubke and R. Mooney (2009). "Avian nucleus retroambigualis: Cell types and projections to other respiratory-vocal nuclei in the brain of the zebra finch (Taeniopygia guttata)." Journal of Comparative Neurology **512**(6): 768-783.

Wild, J. and M. Williams (2000). "Rostral wulst in passerine birds. I. Origin, course, and terminations of an avian pyramidal tract." Journal of Comparative Neurology **416**(4): 429-450.

Wild, J. M. (1987). "Thalamic projections to the paleostriatum and neostriatum in the pigeon (Columba livia)." Neuroscience **20**(1): 305-327.

Wild, J. M. (1988). "Vestibular projections to the thalamus of the pigeon: an anatomical study." Journal of Comparative Neurology **271**(3): 451-460.

Wild, J. M. (1989). "Avian somatosensory system: II. Ascending projections of the dorsal column and external cuneate nuclei in the pigeon." Journal of Comparative Neurology **287**(1): 1-18.

Wild, J. M. (1992). "Direct and indirect "cortico"-rubral and rubro-cerebellar cortical projections in the pigeon." J Comp Neurol **326**(4): 623-636.

Wild, J. M. (1992). "Direct and Indirect Corticorubral and Rubrocerebellar Cortical Projections in the Pigeon." Journal of Comparative Neurology **326**(4): 623-636.

Wild, J. M. (1993). "The avian nucleus retroambigualis: a nucleus for breathing, singing and calling." Brain research **606**(2): 319-324.

Wild, J. M. (1997). "The avian somatosensory system: the pathway from wing to Wulst in a passerine (Chloris chloris)." Brain research **759**(1): 122-134.

Wild, J. M., J. B. Cabot, D. H. Cohen and H. Karten (1979). "Origin, course and terminations of the rubrospinal tract in the pigeon (Columba livia)." Journal of Comparative Neurology **187**(4): 639-654.

Wild, J. M. and S. M. Farabaugh (1996). "Organization of afferent and efferent projections of the nucleus basalis prosencephali in a passerine, Taeniopygia guttata." The Journal of comparative neurology **365**(2): 306-328.

Wild, J. M. and M. N. Williams (1999). "Rostral wulst of passerine birds: II. Intratelencephalic projections to nuclei associated with the auditory and song systems." Journal of Comparative Neurology **413**(4): 520-534.

Wild, J. M., M. N. Williams and R. A. Suthers (2000). "Neural pathways for bilateral vocal control in songbirds." Journal of Comparative Neurology **423**(3): 413-426.

Williams, H. (2001). "Choreography of song, dance and beak movements in the zebra finch (Taeniopygia guttata)." Journal of Experimental Biology **204**(20): 3497-3506.

Winn, P., D. I. G. Wilson and P. Redgrave (2010). "Subcortical Connections of the Basal Ganglia." Handbook of Basal Ganglia Structure and Function **20**: 397-408.

Woolley, S. C. (2016). "Social context differentially modulates activity of two interneuron populations in an avian basal ganglia nucleus." Journal of Neurophysiology **116**(6): 2831-2840.

Woolley, S. M. and E. W. Rubel (1997). "Bengalese finches Lonchura Striata domestica depend upon auditory feedback for the maintenance of adult song." J Neurosci **17**(16): 6380-6390.

Wylie, D. R. (2013). "Processing of visual signals related to self-motion in the cerebellum of pigeons." Frontiers in behavioral neuroscience **7**: 4.

Wylie, D. R., R. Glover and K. Lau (1998). "Projections from the accessory optic system and pretectum to the dorsolateral thalamus in the pigeon (Columbia livia): a study using both anterograde and retrograde tracers." The Journal of comparative neurology **391**(4): 456-469.

Xu, F. and D. T. Frazier (1997). "Respiratory-related neurons of the fastigial nucleus in response to chemical and mechanical challenges." Journal of Applied Physiology **82**(4): 1177-1184.

Xu, F. and D. T. Frazier (2002). "Role of the cerebellar deep nuclei in respiratory modulation." The Cerebellum **1**(1): 35-40.

Xu, F. D., Z. Zhang and D. T. Frazier (2001). "Microinjection of acetazolamide into the fastigial nucleus augments respiratory output in the rat." Journal of Applied Physiology **91**(5): 2342-2350.

Xu, F. D., T. R. Zhou, T. Gibson and D. T. Frazier (2001). "Fastigial nucleus-mediated respiratory responses depend on the medullary gigantocellular nucleus." Journal of Applied Physiology **91**(4): 1713-1722.

Xu, Z., C. Wilson and P. Emson (1991). "Restoration of thalamostriatal projections in rat neostriatal grafts: an electron microscopic analysis." Journal of comparative neurology **303**(1): 22-34.

Yamanaka, K., Y. Hori, T. Minamimoto, H. Yamada, N. Matsumoto, K. Enomoto, T. Aosaki, A. M. Graybiel and M. Kimura (2017). "Roles of centromedian parafascicular nuclei of thalamus and cholinergic interneurons in the dorsal striatum in associative learning of environmental events." Journal of Neural Transmission: 1-13.

Yanagihara, S. and N. A. Hessler (2006). "Modulation of singing-related activity in the songbird ventral tegmental area by social context." European Journal of Neuroscience **24**(12): 3619-3627.

Zackheim, J. and E. Abercrombie (2005). "Thalamic regulation of striatal acetylcholine efflux is both direct and indirect and qualitatively altered in the dopamine-depleted striatum." Neuroscience **131**(2): 423-436.

Zheng, Y. and L. Schwabe (2015). Synaptic amplification by axo-shaft synapses in a pyramidal neuron model. Neural Networks (IJCNN), 2015 International Joint Conference on, IEEE.

Ziegler, W. (2002). "Task-related factors in oral motor control: Speech and oral diadochokinesis in dysarthria and apraxia of speech." Brain and Language **80**(3): 556-575.

Zuschratter, W. and H. Scheich (1990). "Distribution of choline acetyltransferase and acetylcholinesterase in the vocal motor system of zebra finches." Brain research **513**(2): 193-201.