The Role of the Basal Ganglia in Executing and Learning Complex Motor Sequences

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Raymond Ko

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Advisor: Bence Ölveczky Raymond Ko

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Abstract

We learn and perform precise motor sequences to interact with the environment. This ability underlies much of what we do, from playing musical instruments and using new tools to producing fluent speech. Understanding the neural circuits involved in producing these sequences is a central objective of the field of motor learning.

In this dissertation, I study the role of the basal ganglia in complex motor sequence learning and execution, and how they coordinate with the rest of the brain to fulfill both functions. First, I investigate whether the striatum is involved in complex sequence execution by lesioning the dorsolateral striatum (DLS, or sensorimotor striatum) and the dorsomedial striatum (DMS, or associative striatum) in rats trained to execute spatiotemporally precise lever-pressing sequences. Kinematics analysis revealed that DLS lesions significantly disrupted performance, while the DMS was largely dispensable for executing the motor skill. Next, I examined the role of the basal ganglia output in the same task by lesioning the globus pallidus interna (GPi). Third, I explored the role of the DLS and DMS in learning by lesioning the structures prior to training. DLS lesions severely disrupted learning in the task, whereas DMS lesions did not abort learning. Lastly, I examined the role of primary and secondary motor cortices in tutoring the basal ganglia by lesioning them before training. Both cortices have, to at least a degree, redundant functions with respect to learning the task. Overall, this dissertation suggests that the sensorimotor part of the basal ganglia is critical for both executing and learning complex motor sequences.

Table of Contents

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Chapter 1 Introduction

Motivation for studying neural circuits underlying learned motor sequences

We learn and perform precise motor sequences to skillfully interact with our environment. This ability underlies much of what we do, from playing musical instruments and using new tools to producing fluent speech. To perform these activities, we must learn to correctly order the movements of our body parts and optimize their timing (Lashley 1951; Bernstein 1967). Understanding the neural mechanisms involved is a central objective of the field of motor learning (Hikosaka et al. 2002).

Motor sequences are commonly divided into innate and learned ones (Hikosaka 1994; Grillner and Wallén 2004). Innate sequences are largely independent of learning and thought to be underlined by innately wired neural circuits. Some hoofed mammals can stand and walk minutes after birth (Garwicz, Christensson, and Psouni 2009), and human babies can grasp objects forcefully without practice (Twitchell 1965). Other examples include vital movements such as chewing, swallowing and respiration (von Euler 1983; Gray et al. 2001; Lund 1991). In contrast, learned motor sequences require learning and practice as the name implies. They include sequences which are more complex and require fine motor control like speech and dance (Lashley 1951; Bernstein 1967). How the two categories of motor sequences map onto the motor circuitry is not well understood (Grillner and Wallén 2004).

A prevailing view is that learned sequences rely more on cortical circuits whereas innate behaviors depend on subcortical circuits. Behavioral and electrophysiological experiments reveal that motor cortex has a critical role in fine motor control (Romanovich Luria 1966; Tanji and Mushiake 1996; Shima and Tanji 2000). Motor cortex is also required for learning sequences of lever presses in rodents (Kawai et al. 2015; Yin 2009). On the other hand, innate motor sequences such as breathing (von Euler 1983; Gray et al. 2001), swallowing (Lund 1991) and locomotion (Orlovsky, Deliagina, and Grillner 1999; Mori 1992; Rossignol 2010; Grillner 2011) have been found to depend largely on circuits in the brainstem and spinal cord.

Animal studies on grooming have shown a dependence on the basal ganglia (Berridge and Fentress 1987; Pellis et al. 1993; Van Den Bercken and Cools 1982), but not on cortex (Berridge and Whishaw 1992; Whishaw and Kolb 1985). However, as suggested by Grillner and Wallén, insistence on separating innate and learned movements can create a false dichotomy, given that innate movements are also subject to modification and learning (Grillner and Wallén 2004). Moreover, learned motor sequences can be theoretically created by re-arranging pre-established subcortical motor elements (Grillner and Wallén 2004; Shmuelof and Krakauer 2011; Yin 2009).

My research focuses on learned motor sequences, knowing that the hallmark of human motor performance is to acquire and perform complex task-specific motor sequences with practice. Specifically, I study the role of the basal ganglia in complex motor sequence learning and execution, with the aim of deciphering how they coordinate with the rest of the brain to implement their functions. This introductory chapter will provide a review on the role of the mammalian motor system, particularly motor cortex and the basal ganglia, in learning and execution of motor sequences.

The mammalian motor system

Knowledge of the mammalian motor system informs the logic of my approach and the experimental design described in my dissertation. Below, I briefly survey the anatomy of the mammalian motor system and the potential players thought to be relevant to learned motor sequences.

The mammalian brain contains several motor 'controllers' that project directly to the spinal cord to produce movements. At the subcortical level, various brainstem nuclei are major controllers. The mesencephalic and diencephalic locomotor regions (MLR and DLR) control spinal central pattern generators (CPGs) via the reticulospinal pathway (Orlovsky, Deliagina, and Grillner 1999; Grillner 2011),

while the red nucleus coordinates limb movements for gaits and grasping (Muir and Whishaw 2000; Evans and Ingram 1939; Jarratt and Hyland 1999; Martin and Ghez 1991). CPGs for breathing, sucking and chewing also exist in the brainstem (von Euler 1983; Gray et al. 2001; Lund 1991). At the cortical level, motor cortex is the main motor controller. Via the corticospinal tract, it sends projections to the spinal cord (Cheney and Fetz 1980; Fetz et al. 1989), essential for producing complex digit and joint movements (Alaverdashvili and Whishaw 2008; Lawrence and Hopkins 1976; Lemon 1993; Passingham, Perry, and Wilkinson 1983; Whishaw 2000). Besides that, motor cortex influences brainstem CPGs and basal ganglia through corticobulbar tract (Kuypers 1958) and corticostriatal projections (Shepherd 2013; Doig, Moss, and Bolam 2010) respectively.

Other motor-related brain regions can exert modulatory or other effects on motor controllers by providing direct or indirect input to these. The most prominent ones include the basal ganglia, cerebellum and other cortical regions (Kandel 1999). Particularly, the basal ganglia are thought to be involved in multiple sensorimotor functions, including motor skill learning (Hikosaka et al. 1999; Yin, Mulcare, Hilário, et al. 2009) and organization of motor sequences (Benecke et al. 1987a; Graybiel 1998). Anatomical studies have suggested that the basal ganglia form parallel loops with other brain structures (Figure 1.1A). These include the cortical-basal ganglia-thalamic-cortical loops (Figure 1.1B) (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995) and the brainstem-thalamic-basal ganglia-brainstem loops (Figure 1.1C) (Mchaffie et al. 2005; Nandi et al. 2002; Redgrave and Coizet 2007). The architecture of loops likely provides a solution to computational problems in action selection and reinforcement learning, as suggested by previous theoretical work (Hikosaka et al. 2002; Ito and Doya 2011). However, more evidence from animal studies is required to establish the function of the basal ganglia before the theories can be proven and refined.

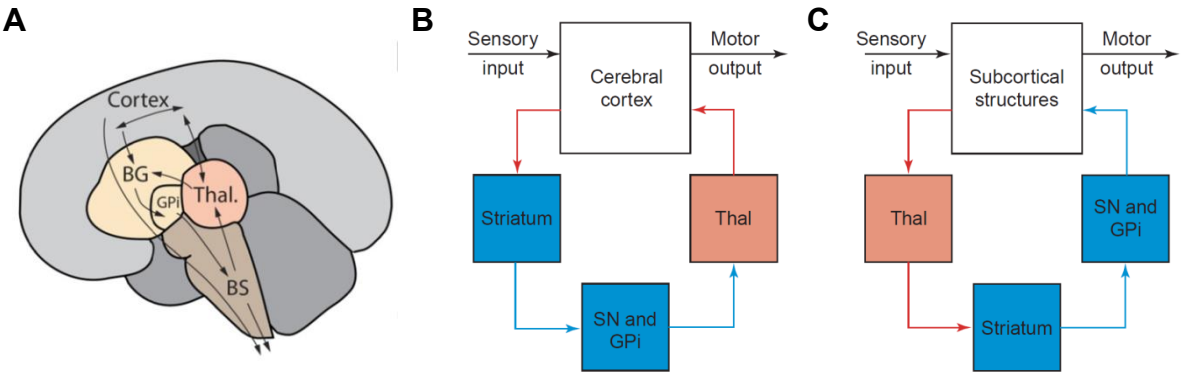

Figure 1.1 Basal ganglia forms parallel loops with other brain structures (Adopted from colleagues in Ölveczky lab and Mchaffie et al. 2005) (A) Schematic showing loop architecture between motor areas. (B) The cortical loop, or the cortico-basal ganglia-thalamic-cortical loop. (C) The subcortical loop, or the brainstem-thalamic-basal ganglia-brainstem loop. Predominantly excitatory regions and connections are in red. Inhibitory ones are in blue. Abbreviations: BG, basal ganglia; BS, brainstem; GPi, internal segment of globus pallidus; SN, substantia nigra; Thal, thalamus.

Given that much of the work on learned motor sequences deals with motor cortex and the basal ganglia, this introductory chapter will mainly focus on these two structures.

The role of motor cortex in motor sequence learning and execution

My dissertation project has to a large extent been inspired by the work on the neural circuits underlying complex motor skills in zebra finches and rodents. Here I will describe how the work of my colleagues in the lab and beyond has contributed to the understanding of cortical and subcortical involvement in motor skill learning.

Motor skill learning and execution are commonly believed to rely on motor cortex. Neurons in motor cortex have been found to encode various motor parameters, including direction (Georgopoulos et al. 1982; Georgopoulos, Schwartz, and Kettner 1986; Kettner, Schwartz, and Georgopoulos 1988; Georgopoulos, Kettner, and Schwartz 1988; Schwartz, Kettner, and Georgopoulos 1988), force (Cheney and Fetz 1980; Fetz et al. 1989; Georgopoulos et al. 1992), distance (Fu, Suarez, and Ebner 1993; Fu et al.

1995), anticipation (Tanji and Evarts 1976; Georgopoulos, Kettner, and Schwartz 1988; Ashe et al. 1993; Lu and Ashe 2005) and goals (Alexander and Crutcher 1990; Kakei, Hoffman, and Strick 1999).

Moreover, microstimulation of motor cortex for long durations not only triggers simple movements (Ferrier 1875; Campbell 1905) but can also elicit more complex, ethologically relevant motor sequences (Graziano, Taylor, and Moore 2002; Graziano 2006; Graziano and Aflalo 2007; Graziano, Aflalo, and Cooke 2005). Motor cortex is also found to be involved in learned sequences of finger movements (Ungerleider, Doyon, and Karni 2002; Pascual-Leone, Grafman, and Hallett 1994; Seidler et al. 2005; Doyon et al. 2009). Furthermore, skill learning can be impaired by transcranial magnetic stimulation of motor cortex (Pascual-Leone et al. 1995; Gerloff 1998; Richardson et al. 2006). Finally, spinal projections from motor cortex are implicated in executing independent digit and joint movements, or 'dexterity' (Alaverdashvili and Whishaw 2008; Lawrence and Hopkins 1976; Lemon 1993; Passingham, Perry, and Wilkinson 1983; Whishaw 2000).

Surprisingly, however, our lab discovered that motor cortex is not required for executing learned complex motor sequences that do not have dexterity requirements (Kawai et al. 2015). Specifically, we developed a paradigm that allows us to look at motor sequence learning in rodents (Detailed method to be described in Chapter 2). By operant conditioning, rats are trained to press a lever twice with a precise inter-press interval (IPI) of 700ms (Figure 1.2A). Superstitious movements like nodding and limb flicking are often incorporated into final motor sequences. As a result of many weeks of trial-and-error learning, the animals perform complex, spatiotemporally precise motor sequences. Pharmacological lesion of motor cortex, remarkably, impaired neither the timing of the presses (Figure 1.2B) nor the associated paw kinematics (Figure 1.2C), implicating subcortical structures in the storage and execution of these motor skills.

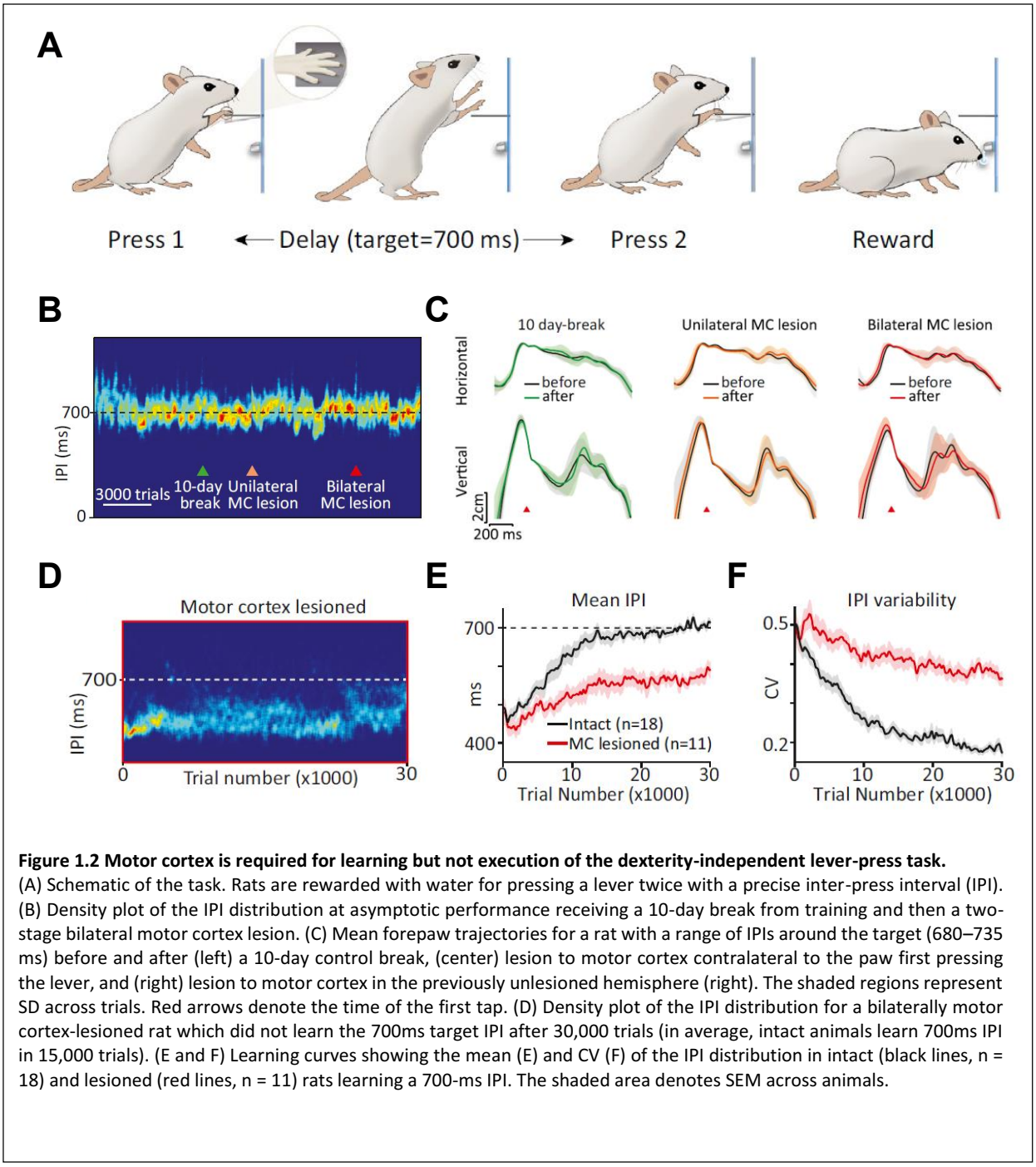

Figure 1.2 Motor cortex is required for learning but not execution of the dexterity-independent lever-press task.
(A) Schematic of the task. Rats are rewarded with water for pressing a lever twice with a precise inter-press interval (IPI). (B) Density plot of the IPI distribution at asymptotic performance receiving a 10-day break from training and then a two-stage bilateral motor cortex lesion. (C) Mean forepaw trajectories for a rat with a range of IPIs around the target (680–735 ms) before and after (left) a 10-day control break, (center) lesion to motor cortex contralateral to the paw first pressing the lever, and (right) lesion to motor cortex in the previously unlesioned hemisphere (right). The shaded regions represent SD across trials. Red arrows denote the time of the first tap. (D) Density plot of the IPI distribution for a bilaterally motor cortex-lesioned rat which did not learn the 700ms target IPI after 30,000 trials (in average, intact animals learn 700ms IPI in 15,000 trials). (E and F) Learning curves showing the mean (E) and CV (F) of the IPI distribution in intact (black lines, n = 18) and lesioned (red lines, n = 11) rats learning a 700-ms IPI. The shaded area denotes SEM across animals.

In the same study, we found that motor cortex is required for learning the exact same task. Motor cortex lesion prior to training impaired the ability of animals to learn the 700ms IPI under the same training paradigm (Figures 1.2D and E). Temporal variability remained high as compared with intact

animals (Figure 1.2F). These results suggest that subcortical circuits can autonomously execute learned motor sequences and that motor cortex may 'tutor' subcortical circuits during trial-and-error learning.

But if motor cortex is not necessary for execution, then which subcortical controllers are involved? And how are these controllers 'tutored' by motor cortex? These are the two central questions to be explored and discussed in this dissertation.

The role of the basal ganglia in motor sequence learning and execution

Neuroanatomical and clinical evidence has inspired me to select the basal ganglia as my first study target. First, around 50% of projections received by the basal ganglia comes from the cortex (Doig, Moss, and Bolam 2010). Second, the basal ganglia are part of both cortical (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995) and subcortical (Mchaffie et al. 2005; Nandi et al. 2002; P. Redgrave and Coizet 2007) loops, which have been implicated in movement selection and thus sequence generation (Hikosaka et al. 2002; Ito and Doya 2011). Third, basal ganglia diseases such as Parkinson's lead to deficits in sequence learning and execution (Vakil et al. 2000; Brown et al. 2003; Weiss, Stelmach, and Hefter 1997; Benecke et al. 1987a; Agostino et al. 1992). Therefore, there is good prior evidence for the basal ganglia serving as a subcortical substrate for learned motor sequences.

The role of the basal ganglia in motor sequence execution

In rats, striatal lesion disrupts the execution of action sequences associated with grooming and play fighting, which are examples of well-characterized innate motor sequences in rodents (Berridge and Fentress 1987; Pellis et al. 1993). Similarly, pharmacological manipulations of striatum in primates disrupt the execution of naturally expressed sequential behaviors (Van den Bercken and Cools 1982). Similar results have been seen for learned motor sequences. In a sequential button-press task in primates,

muscimol inactivation of striatum led to higher error rates in execution of learned motor sequences in a visuomotor task (Miyachi et al. 1997). Likewise, dopamine depletion in striatum, which has a preponderance of dopaminergic synapses, disrupted learned motor sequences (Matsumoto et al. 1999).

One confound in these studies is that a loss of neurons after striatal lesions might cause aberrant neural activity, which in turn perturbs processing in other motor areas. These dysfunctions are routinely observed in Parkinson's and Huntington's patients, in which partial loss of neurons can be found in the basal ganglia (Hammond, Bergman, and Brown 2007; Jenkinson and Brown 2011; Marreiros et al. 2013; Uhlhaas and Singer 2006; Mink 1996). Such loss of neurons could lead to the imbalance of excitatory and inhibitory activities, resulting in pathological firing. Pathological firing in the basal ganglia could disrupt the normal neural activity of the basal ganglia-recipient motor controllers in brainstem and motor cortex, leading to the defects observed in striatal lesion studies. Such defects may thus not reflect loss-of-function in the basal ganglia (Ayalon et al. 2004; Shin, Aparicio, and Ivry 2005). That pallidotomies (i.e. lesions to the basal ganglia output), which effectively block aberrant firing patterns in the basal ganglia relieve motor-related symptoms of striatal dysfunction support this view (Mink 1996; Vitek and Giroux 2000; Okun and Vitek 2004).

Interestingly, lesion or inactivation of the GPi produce only subtle motor deficits in rodents (Lütjens et al. 2011), humans (Bhatia and Marsden 1994; Obeso et al. 2009) and monkeys (Desmurget and Turner 2010). In contrast to striatal manipulations mentioned above, muscimol inactivation of GPi in trained monkeys did not impair sequencing movements in a visuomotor task (Desmurget and Turner 2010). This supports the idea that the basal ganglia are not required for motor sequences explicitly learned through instructional cues. However, whether the basal ganglia are required for sequences learned without instructional cues (i.e. 'implicit learning') has not yet been resolved.

Besides animal experiments, patients with Parkinson's disease also provide insights into how the basal ganglia are involved in movement execution. Parkinsonian patients often show slowness and fatigue when they perform ordinary chores. Such deficit is not only caused by muscle rigidity and tremor but also inability in performing multiple sequences simultaneously (Schwab, Chafetz, and Walker 1954). Furthermore, patients have difficulty in performing rapid sequential movements due to the increase in movement duration and difficulty in switching from one movement to the next (Agostino et al. 1992; Benecke et al. 1987a). All of these suggest that the basal ganglia play an important role in complex motor sequence execution in humans. However, in Parkinsonian patients, aberrant activity from the basal ganglia spreads to other motor areas and induces wide range of motor deficits (Hammond, Bergman, and Brown 2007; Jenkinson and Brown 2011; Marreiros et al. 2013; Uhlhaas and Singer 2006; Mink 1996), making it difficult to draw definitive conclusions relating to the role of different basal ganglia nuclei.

The role of the basal ganglia in motor sequence learning

The basal ganglia have not only been implicated in the execution of motor sequences, but also independently in their acquisition. Striatal activity is dynamically modified in rodents during learning of motor skills and habit formation (Yin et al. 2009; Barnes et al. 2005; Costa et al. 2004). In functional imaging studies, striatum was activated during motor sequence learning (Doyon et al. 2006; Grafton, Hazeltine, and Ivry 1995; Rauch et al. 2004; Rauch et al. 1998). Moreover, some Parkinsonian patients show deficits in sequence learning (Pascual-Leone et al. 1993; Jackson et al. 1995; Helmuth, Mayr, and Daum 2000).

Different neural substrates may be required for early and late stages of motor learning. Miyachi et al (2002) showed that neurons which were activated preferentially for new motor sequences were mainly located in the associative striatum, while those for learned sequences were more abundant in the sensorimotor striatum. This is consistent with an earlier study in which inactivation of sensorimotor

striatum led to learned motor sequences being more affected as compared with inactivation of associative striatum (Miyachi et al. 1997). Also, an fMRI study on human subjects who were trained to perform sequential finger movements showed that activation increases with practice in sensorimotor striatum but decreases in associative striatum (Coynel et al. 2010; Lehericy et al. 2005). However, most of the above studies involved only electrophysiological or functional imaging experiments. More lesion and inactivation studies in animals are critical to establish the casual link between neural activity of the basal ganglia and their motor functions.

Furthermore, many of these studies on motor sequences did not look at temporally precise sequences, nor did they attempt to train animals to complete the sequences within a specific duration. The basal ganglia have been hypothesized to be important for temporally precise motor sequences, as implied by clinical observations showing that Parkinson's and Huntington's patients are poor at temporal organization of speech (Ludlow, Connor, and Bassich 1987; Volkmann et al. 1992). Yet animal studies have rarely been performed to answer the question, possibly because training of precise motor sequences requires considerable amount of time and effort.

The birdsong model provides a solution to the above problem. The zebra finch has long been a model organism for motor sequence learning and execution due to the precise and complex spectral/temporal structure of its songs (Marler 2004; Doupe and Kuhl 1999), ease of quantifying the behavior (Lipkind and Tchernichovski 2011; Tchernichovski et al. 2000) and specific cortical and subcortical circuits dedicated to song learning (Doupe et al. 2005; Vu, Mazurek, and Kuo 1994; Nottebohm, Kelley, and Paton 1982; Wild, Williams, and Suthers 2000). My colleague Farhan Ali found that a pre-motor cortical area encodes temporal structure of birdsong, while the basal ganglia are required for learning spectral structure (Ali et al. 2013). The experiment was based on a recently introduced technique, conditional auditory feedback (CAF) (Tumer and Brainard 2007; Andalman and and Fee 2009), in which auditory feedback is provided contingent on certain attributes of the song (e.g. the pitch or duration of a

syllable) to adaptively modify the song. For instance, the pitch of a syllable can be shifted higher by providing aversive white noise auditory feedback whenever a bird sings a low-pitch syllable. After bilateral lesions of Area X, the avian striatal-pallidal analog (Farries, Ding, and Perkel 2005; Farries and Perkel 2002; Carrillo and Doupe 2004; Goldberg and Fee 2010), learning in the spectral domain (pitch) was abolished (Ali et al. 2013). While Area X lesions disrupt learning, they do not interfere with execution of learned song. These studies motivated the lab to explore the contributions of the mammalian basal ganglia to motor sequence learning and execution.

Given the above discussion, the role of the basal ganglia in sequence learning and execution is far from resolved. To parse the issue further, we developed an experimental paradigm that overcomes the drawbacks of previous studies, and investigated whether the basal ganglia are important for learning and executing complex motor sequences. Key distinctions of our paradigm include the following: (1) Our motor task has been verified to be cortex-independent (Kawai et al. 2015), and so any effect of our lesions will implicate subcortical basal ganglia loops; (2) Lesions were performed in a precise, controlled manner pharmacologically yielding cleaner results as compared with studies on basal ganglia strokes and Parkinson's Disease; (3) GPi lesion in addition to striatal lesion was performed to ensure loss-of-function was not caused by the spread of aberrant activity in striatum (Mink 1996; Vitek and Giroux 2000; Okun and Vitek 2004); (4) Rodents as a mammalian model provide insights potentially more applicable to humans as compared with non-mammalian vertebrates (Anton Reiner, Brauth, and Karten 1984; Reiner, Medina, and Veenman 1998; Smeets, Marín, and González 2000).

Neuroanatomy of the basal ganglia

Here I provide a brief overview the anatomy of the mammalian basal ganglia circuit, which will inform the logic underlying my experimental approach and my hypotheses.

Basal ganglia connections

Basal ganglia consist of a set of interconnected subcortical nuclei (Figure 1.3) that are involved in multiple sensorimotor functions, including motor skill learning (Hikosaka et al. 1999; Yin, Mulcare, Hilario, et al. 2009) and organization of motor sequences (Benecke et al. 1987b; Graybiel 1998). Among its nuclei, striatum is the largest one in both rodents and primates (Hardman et al. 2002; Beckmann and Lauer 1997), and it receives inputs mostly from cortex and thalamus (Doig, Moss, and Bolam 2010), potentially integrating motor and contextual information from both structures for movement execution (Fee 2012). Striatal neurons also receive dopaminergic input from substantia nigra pars compacta, which may be excitatory or inhibitory depending on the dopamine receptor expressed in the postsynaptic striatal neurons (Redgrave and Gurney 2006; Gerfen and Surmeier 2011).

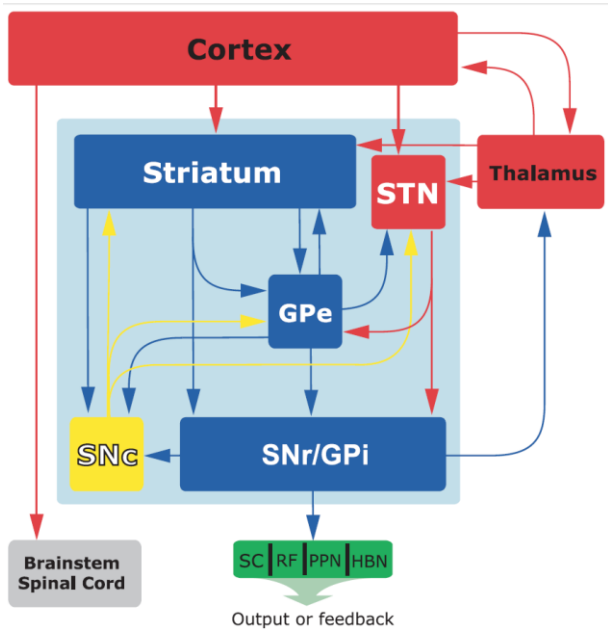

Figure 1.3 Schematic of the basal ganglia circuits (adopted from Tepper, Abercrombie, and Bolam 2007).
The nuclei of the basal ganglia are included in the light blue box and consist of the striatum, the substantia nigra pars compacta (SNc), the external segment of the globus pallidus (GPe), the subthalamic nucleus (STN), the substantia nigra pars reticulate and the internal segment of the globus pallidus (SNr/GPi). The two major inputs to the basal ganglia are from the cortex and the thalamus. The basal ganglia influence behavior by the output nuclei SNr/GPi projecting to the thalamus and back to the cortex, and projections to the superior colliculus (SC), the reticular formation (RF), the pedunculopontine nucleus (PPN) and the lateral habenula (HBN). Dopamine neurons of the substantia nigra pars compacta (SNc) provide a massive feedback to the striatum and also the GPe and STN that modulates the flow of cortical and thalamic information through the basal ganglia. Dark blue indicates structures that are principally GABAergic; red indicates structures that are principally glutamatergic, yellow indicates structures that are dopaminergic and green indicates basal ganglia targets.

The internal segment of globus pallidus (GPi) and the substantial nigra pars reticulata (SNr) serve as the major output nuclei of the basal ganglia. They project to subcortical motor areas like the pedunculopontine nucleus of brainstem, multiple regions of the thalamus, including the motor thalamus

and parafascicular nucleus (Pf), and the lateral habenula (Van Der Kooy and Carter 1981; Takada et al. 1994; Carter and Fibiger 1978). Traditionally, these projection neurons were thought to be mostly tonically firing GABAergic neurons, inhibiting downstream areas. However, by means of optogenetics stimulation and neurochemistry, Shabel et al. discovered glutamatergic excitatory projections from GPi to lateral habenula (Shabel et al. 2012), while Kha et al. found that approximately half of the GPi-thalamic projection neurons are cholinergic (Kha et al. 2000). Nevertheless, the function of these excitatory projections in movement execution remains unclear.

Direct and indirect pathways

In between the input and output nuclei of the basal ganglia, two pathways have been identified and widely recognized. They are the direct and indirect pathways, which form the basis of the action-selection theory (see Groenewegen 2003; Redgrave, Prescott, and Gurney 1999 for review). Both pathways originate from striatal neurons. In the direct pathway, inhibitory striatal neurons expressing GABA plus dynorphin and neuroactive peptides substance P (SP) project monosynaptically to GPi/SNr (Gerfen and Wilson 1996). These neurons reduce the tonic inhibition exerted by GPi/SNr neurons on their downstream motor areas and trigger movements. In the indirect pathway, another group of inhibitory striatal neurons, biochemically identified with GABA and encephalin, polysynaptically project to and inhibit GPi/SNr via the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN) (Gerfen and Wilson 1996). As a result, this pathway excites the inhibitory GPi/SNr neurons and thus is thought to inhibit movements (Smith et al. 1998). Therefore, to initiate a movement, the direct pathway must counteract the indirect pathway and disinhibit the control circuits that generate the specific movement, while the indirect pathway continues to inhibit other undesired movements (Mink 1996; Wickens 1997).

Direct and indirect pathways are not the only pathways in the basal ganglia exerting influences over downstream motor areas. The cortico–subthalamo–pallidal ‘hyperdirect’ pathway (i.e. cortex – STN

– GPi/SNr) bypasses the striatum and inhibits downstream motor systems with a shorter conduction time (Nambu, Tokuno, and Takada 2002). The 'center surround model' (Mink and Thach 1993; Mink 1996; Hikosaka, Takikawa, and Kawagoe 2000) suggest that this pathway ensures better temporal precision of movement initiation. The idea is that before the direct pathway selects a desired movement, the hyperdirect pathway first inhibits the other motor programs globally to prevent undesired movements from being accidentally triggered by the excitatory effects of the direct pathway. However, proving this hypothesis experimentally could be challenging, given that manipulation of STN would affect both the hyperdirect and indirect pathways simultaneously.

Cortical and subcortical loops

The basal ganglia form parallel loops with other brain regions as discussed earlier (Figure 1.1A). One important example is the cortical-basal ganglia-thalamic-cortical loops (Alexander, DeLong, and Strick 1986; Parent and Hazrati 1995; Middleton and Strick 2000) (Figure 1.1B), which have been suggested to have a role in action selection and reinforcement learning (Hikosaka et al. 2002; Ito and Doya 2011).

One hallmark of the cortical-basal ganglia-thalamic-cortical loops is that they can be further segregated into parallel pathways according to their anatomy and functions. Two pathways have been widely recognized based on neuroanatomical data:

1) The "*motor loop*" includes the sensorimotor striatum (the dorsolateral striatum or DLS in rodents) mainly receives projections from motor and somatosensory cortices,

2) The "*association loop*" passing through the associative striatum (the dorsomedial striatum or DMS in rodents) receives projections from prefrontal and posterior parietal cortices.

The differences between DLS and DMS are described in the Introduction to Chapter 2. Within these loops, functionally segregated regions of cortex project to corresponding regions of the basal

ganglia and thalamus and then return to the cortex (Alexander, DeLong, and Strick 1986; Parent and Hazrati 1995; Middleton and Strick 2000). However, the loops are not necessarily rigidly closed loops without communications with other loops or brain structures. For example, some projections from GPi to motor thalamus simultaneously project to the habenula and brainstem (Kha et al. 2000), allowing information outflow from the loops. Also, interconnections between different cortical regions (Reep, Goodwin, and Corwin 1990; Jacobs and Donoghue 1991) potentially permit interactions between the loops.

Besides the cortical loops, another circuit which involves the basal ganglia is the brainstem-thalamic-basal ganglia-brainstem loop, or the subcortical loop (Figure 1.1C) (Mchaffie et al. 2005; Nandi et al. 2002; Redgrave and Coizet 2007). Various movement-related brainstem structures provide inputs to the midline intralaminar complex of the thalamus which projects to the striatum. The basal ganglia output nuclei, GPi and SNr, then link back to the brainstem structures accordingly (Redgrave, Marrow, and Dean 1992; Takada et al. 1994). These brainstem structures include (but are not limited to) the superior and inferior colliculi (Krout et al. 2001; Krout, Belzer, and Loewy 2002), pedunculopontine nucleus (Erro, Lanciego, and Giménez-Amaya 1999), periaqueductal grey (Krout and Loewy 2000) and multiple pontine and medullary reticular nuclei (Krout, Belzer, and Loewy 2002). This phylogenetically older subcortical loop involving brainstem motor areas could facilitate action selection in vertebrates that rely on their subcortical motor infrastructure for generating movements (Grillner and Robertson 2015; Grillner et al. 2005).

Overview of dissertation chapters

To recapitulate the central questions in this dissertation: Is the basal ganglia circuit an essential subcortical substrate for the generation of complex motor sequences? If so, which parts of the basal ganglia are required for execution and learning? And how is the motor cortical input to the striatum modulating basal ganglia function?

In Chapter 2, I address the question of how the striatum is involved in complex sequence execution by lesioning DLS and DMS in rats trained to execute spatiotemporally precise motor sequences, and compare movement kinematics and motor sequences before and after lesion. After delineating the role of DLS in execution, Chapter 3 describes the role of the basal ganglia output in the same task by lesioning GPi. Chapter 4 moves on to describe the role of DLS and DMS in learning by lesioning the respective structures prior to training. Finally, Chapter 5 examines the role of primary and secondary motor cortices in tutoring by lesioning them before training. Hypotheses on the neural circuits involved in executing and learning complex motor sequences are formulated based on the above results in the last chapter of the dissertation.

Chapter 2 Role of the striatum in motor sequence execution

Introduction

To understand the role of the basal ganglia in the execution of learned motor sequences, I first focused on the striatum, which receives inputs from both the cortex and the thalamus (Shepherd 2013; Doig, Moss, and Bolam 2010), and is thus well situated for integrating motor and contextual information for motor execution (Fee 2012). Furthermore, striatal lesions are known to interrupt execution of well-characterized innate motor sequences (Berridge and Fentress 1987; Pellis et al. 1993) and visually guided motor sequences (Miyachi et al. 1997; Matsumoto et al. 1999). Finally, the associative (DMS) and sensorimotor (DLS) regions of the striatum are anatomically relatively well separated (McGeorge and Faull 1989; Devan, Hong, and McDonald 2011; Voorn et al. 2004), which has allowed their roles in multiple behavioral tasks to be differentiated (Yin, Knowlton, and Balleine 2004; Yin and Knowlton 2004; Skelin et al. 2014; Hilario et al. 2012; Wang, Miura, and Uchida 2013; Bailey and Mair 2006; Balleine and O'Doherty 2009; White 2009; Yin, Knowlton, and Balleine 2006; Ragozzino 2007). This motivates me to target the striatum for investigating the roles of associative and sensorimotor loops (see review in Chapter 1) in learned motor sequence execution (Desmurget and Turner 2010; Shmuelof and Krakauer 2011).

In chapter 1, I mentioned that basal ganglia disorders provide insights into the role of the basal ganglia, as well as the striatum, in motor sequence execution. In patients with Parkinson's disease, sequence learning and execution are often affected (Vakil et al. 2000; R. G. Brown et al. 2003; Weiss, Stelmach, and Hefter 1997; Benecke et al. 1987a; Agostino et al. 1992). They have difficulty performing different sequences simultaneously (Schwab, Chafetz, and Walker 1954) and transitioning from one movement to another (Agostino et al. 1992; Benecke et al. 1987a). However, one major drawback of these studies is that the location and volume of affected brain areas are not controlled, leading to unexplained variability in motor deficits among patients (Pascual-Leone et al. 1993; Jackson et al. 1995; Helmuth, Mayr,

and Daum 2000). Furthermore, cognitive factors such as motivation and attention complicate these patient studies (Lees and Smith 1983; Fisher et al. 1983; Talland and Schwab 1964; Horne 1973). Localized and precise brain lesions in animals, to be described in the coming chapters, are hence essential to draw cleaner and more grounded conclusions.

The striatum, however, is not a homogenous structure and can be divided according to differences in anatomy and function, as discussed below. The dorsomedial striatum (DMS) receives projections mostly from associative areas of the cortex (e.g., the visual, auditory and prefrontal cortices) and the thalamus (McGeorge and Faull 1989; Voorn et al. 2004; Haber 2003), while the dorsolateral striatum (DLS) is more interconnected with the motor and somatosensory regions. Functionally, the DMS is thought to be critical for goal-directed behaviors (Yin, Knowlton, and Balleine 2005; Yin et al. 2005; Balleine and Dickinson 1998; Balleine and O'Doherty 2009) and initial skill learning (Miyachi et al. 1997; Hikosaka et al. 1995), whereas the DLS is believed to be involved in habit formation (Yin, Knowlton, and Balleine 2004; Yin, Knowlton, and Balleine 2006; White 2009; Balleine and O'Doherty 2009) and the execution of over-trained motor skills (Miyachi et al. 1997; Hikosaka et al. 1995; Graybiel 2008). Physiologically, DMS neurons encode stimulus-reward contingencies (Kimchi et al. 2009; Kimchi and Laubach 2009; Yin et al. 2009), while DLS neurons are modulated by the animal's motor output (Barnes et al. 2005; Kimchi et al. 2009; Yin et al. 2009; Tang et al. 2007; Kubota et al. 2009). So, even though there is not a clear anatomical or biochemical boundary between the DLS and DMS (McGeorge and Faull 1989; Devan, Hong, and McDonald 2011; Voorn et al. 2004), it is still important to treat them as separate entities in experiments.

Strengths of the training system and paradigm

My dissertation takes advantage of a timed lever-pressing task in rodents that trains spatiotemporally precise movement sequences (Kawai et al. 2015). Thus, I will introduce the experimental infrastructure used to train these behaviors and discuss the strengths of the paradigm. Our lab has developed a fully automated experimental platform for training complex behaviors in rats (Poddar, Kawai, and Ölveczky 2013). Automated training in home cages significantly decreases human involvement and makes experiments with large sample numbers effortless, which is important when training and studying slow-to-learn motor behaviors.

Rat as the model organism. The rat has emerged as a mammalian model for complex behaviors (Uchida and Mainen 2003; Blaisdell et al. 2006; Kepecs et al. 2008; Murphy, Mondragón, and Murphy 2008; Zeeb, Robbins, and Winstanley 2009; Zoccolan et al. 2009; Abbott 2010; Viana et al. 2010; Ölveczky 2011). While high-throughput training in non-human primates is difficult due to costs and regulations (Goodman and Check 2002), rats require less space, effort and money to house, allowing simultaneous training of large cohorts. Also, compared to mice, rats can accommodate larger chronically implanted devices, allowing for neural recording of large neuronal populations (Bragin et al. 2000; Hasegawa et al. 2015; Lee et al. 2006) and sophisticated optogenetics manipulations (Boyden 2011; Zalocusky and Deisseroth 2013; Zhang et al. 2010) targeting multiple brain regions in freely behaving animals. Importantly, the basal ganglia are well conserved among mammals, so rat studies can also inspire basal ganglia research in humans (Stephenson-Jones et al. 2011; Grillner and Robertson 2015; Anton Reiner, Brauth, and Karten 1984; Reiner, Medina, and Veenman 1998; Smeets, Marín, and González 2000).

High throughput. High-throughput training is crucial to our studies. We investigated five brain regions in over 40 animals to understand the cortical-basal ganglia circuitry. It took 3 to 8 weeks for an animal to learn a stereotyped motor sequence, potentially adding up to 30,000 trials with an average daily

performance of 500 trials. Having an automated, high-throughput system frees up a tremendous amount of time from training the complex and slow-to-learn task. Also, a high sample number is required for understanding the relationship between the performance degradation and the lesion volume of a targeted brain region.

Standardized training procedure. Animal training is potentially vulnerable to human intervention. For instance, animal behavior is influenced by handling (Hurst and West 2010) or even the gender of the animal handlers (Sorge et al. 2014). Moreover, unconscious bias can be introduced if the handlers are aware of the experimental manipulations performed. Therefore, to ensure objectiveness, intervention should be minimized. This can be achieved by automated home-cage training. Water can be automatically provided if the minimal daily intake is not reached. Animals should only be removed from their home cages for surgery and cage cleaning. Also, the enrichment in training cages should be identical, since it potentially changes brain plasticity and behavioral performance (Diamond et al. 1972; Fares et al. 2013; Harding, Paul, and Mendl 2004). In our case, each cage included a plastic cylinder, a food bowl and a fixed amount of soil.

Automated updates to the training protocol. Complex motor sequence training is a long process of trial-and-error learning. In order to facilitate learning, reward contingency should be gradually changed according to recent performance (as in the video games that humans play). If the performance is better than expected, the reward range will be tightened to push the animal to do even better. If the performance is poorer, the reward range will be loosened so that the animal will not be discouraged and stop working. Therefore, the training system should be capable of automatically retrieving behavioral data, evaluating the performance and adjusting the reward range according to a standard set of rules. Doing all of these actions manually would be extremely tedious and prone to errors (Schaefer and Claridge-Chang 2012; Hurst and West 2010). Therefore, our training system has been designed to be self-contained: It automates all aspects related to animal training from collecting behavioral data and analyzing

performance, to adjusting training parameters. Furthermore, daily performance updates ensure animal welfare and safety. Experimenters are alerted if abnormal changes are observed in training intensity, performance quality or amount of water consumed.

The novel paradigm opens up the possibility of studying complex motor sequences in rodents in a high-throughput and convenient manner. Utilizing this powerful tool allows us to ask the question I stated in Chapter 1: Are the basal ganglia an essential subcortical substrate for acquiring and executing complex motor sequences?

Lesion methodology

Given that the experiments described in the coming chapters rely on pharmacological lesions, I briefly review the pros and cons of the methodology.

Pharmacological lesion has long been a method to establish causality between brain regions and behaviors (Mcgeer, Olney, and Mcgeer 1978). It remains an important technique in neuroscience as functional imaging (Henson 2005; Paus 2005; Brown and Eyler 2006; Weber and Thompson-Schill 2010) and neural recording (Humphrey 2000; Sporns 2010) mainly demonstrate correlation between neural activity and functionality, while it is difficult for other transient manipulations, like optogenetics and electrical/magnetic stimulation, to provide a stable blockade of a specific region for a long time period (Packer, Roska, and Häusser 2013; Butler 2012; Bolognini and Ro 2010; Rossini and Rossi 2007). Furthermore, transient manipulation may have off-target effects on remote brain areas making it difficult to infer the function of the targeted circuit based only on such manipulations (Otchy et al. 2015)

In a pharmacological lesion, neurons of a targeted brain region are killed by injection of neurotoxin into the brain tissue. Examples of neurotoxins include quinolinic acid (Beal et al. 1988;

Schwarcz, Whetsell, and Mangano 1983; Perkins and Stone 1983) and ibotenic acid (Schwarcz et al. 1979; Kohler and Schwarcz 1983), which are potent glutamate receptor agonists that induce excitotoxicity. Importantly, both selectively target cell bodies and spare axons from non-targeted brain regions, bypassing the pitfall of electrolytic lesion (Schwarcz, Whetsell, and Mangano 1983; Coyle 1981). This allows controlled and localized lesions of basal ganglia nuclei.

In our experiments, all bilateral basal ganglia lesions were conducted in two or more separate surgeries to avoid motor deficits. One-stage bilateral striatal lesions lead to severe motor deficits according to our experience, and one-stage bilateral lesions of the internal segment of globus pallidus (GPi) can even be lethal (Merello et al. 2001; Schwabe, Polikashvili, and Krauss 2009). We first lesioned the targeted brain region unilaterally (contralateral to the forepaw used in the first lever press) and then resumed training. If the performance recovered or remained intact, we went on to lesion the ipsilateral side.

In this chapter, we examine the role of the DLS and DMS in the execution of learned motor sequences. After training animals to perform the lever-pressing task according to the paradigm discussed above, we pharmacologically lesioned the targeted region and quantified how these circuit manipulations affected the timing and kinematics of the learned motor sequences.

Methods

Animals

All experiments were conducted in accordance with the protocols approved by the Harvard Institutional Animal Care and Use Committee. Experimental subjects were female Long Evans rats, 4–8-months old at start of training (Charles River Laboratories). Water consumption was restricted to 15 mL per day. Food was available at all times in the home cages.

Behavioral training and testing

Animals were trained to press a lever twice, and only twice. In order to obtain a reward, the time between the two presses (the inter-press interval, or IPI) had to fall within a specified interval. The reward interval was gradually tightened throughout the learning phase and shaped the IPI to 700ms, which is a relatively long period of time as compared with rats' natural inter-press interval (~300ms) (Kawai et al. 2015).

A total of 16 rats were trained in the motor task. Training took place in home cages, which were custom-made operant conditioning boxes separated by light-resistant and sound-attenuating walls (Figure 2.1A). These boxes were supplied with a water spout for reward delivery and a lick sensor for dispensing the reward appropriately, and also a speaker for reward tone generation. It was further outfitted with a webcam (30fps, Agama V-1325R) for monitoring behavior, an air pump for ventilation and a plastic cylinder as enrichment (Figure 2.1C). The lever was placed 14cm above the floor, between a pair of glass protrusions, to ensure that the animal pressed the lever with its forepaws and not with its snout (Figure 2.1D). The force required to press the lever was <0.1N. A deflection of 3mm was required for registering a lever press. Technical details of software and hardware of the fully automated training system have been described in detail (Figure 2.1B, Poddar, Kawai, and Ölveczky 2013).

Water-restricted animals were kept in training cages for the duration of the experiments. A 12-hour daylight cycle was followed. Three 1-hour training sessions were given each day during the animals' subjective "day time" (i.e., lights off), 7 days a week. At the beginning of each session, the house light blinked and the speaker produced beeping sounds temporarily to signify the start of a session. The house light was turned on throughout the training session, and it was turned off at the end of a session. Webcams were used to record movements in each trial. Amount of water delivery was tracked by a computer. By the end of each day, free water was given to the animals if they did not get enough water during training sessions.

Behavioral training started with one to two days of tone conditioning. The animals received a drop of water when they licked the water spout in response to a reward tone. After the animals learned to associate the reward tone with water availability, they received three to four days of one-press training during which the reward tone was given after each lever press to inform the animal of the available water reward.

During two-press training, rats self-initiated a trial by pressing the lever. If the IPI fell outside the rewarded range, animals had to wait 1.2 seconds before initiating the next trial (inter-trial delay). Rats were initially rewarded for pressing the lever twice between 200 and 1100ms. Reward boundaries were then dynamically adjusted to shape the animal's behavior toward the target IPI while maintaining reward rates around 35%.

After surgery, the initial reward range was set to that of the training session immediately before lesioning.

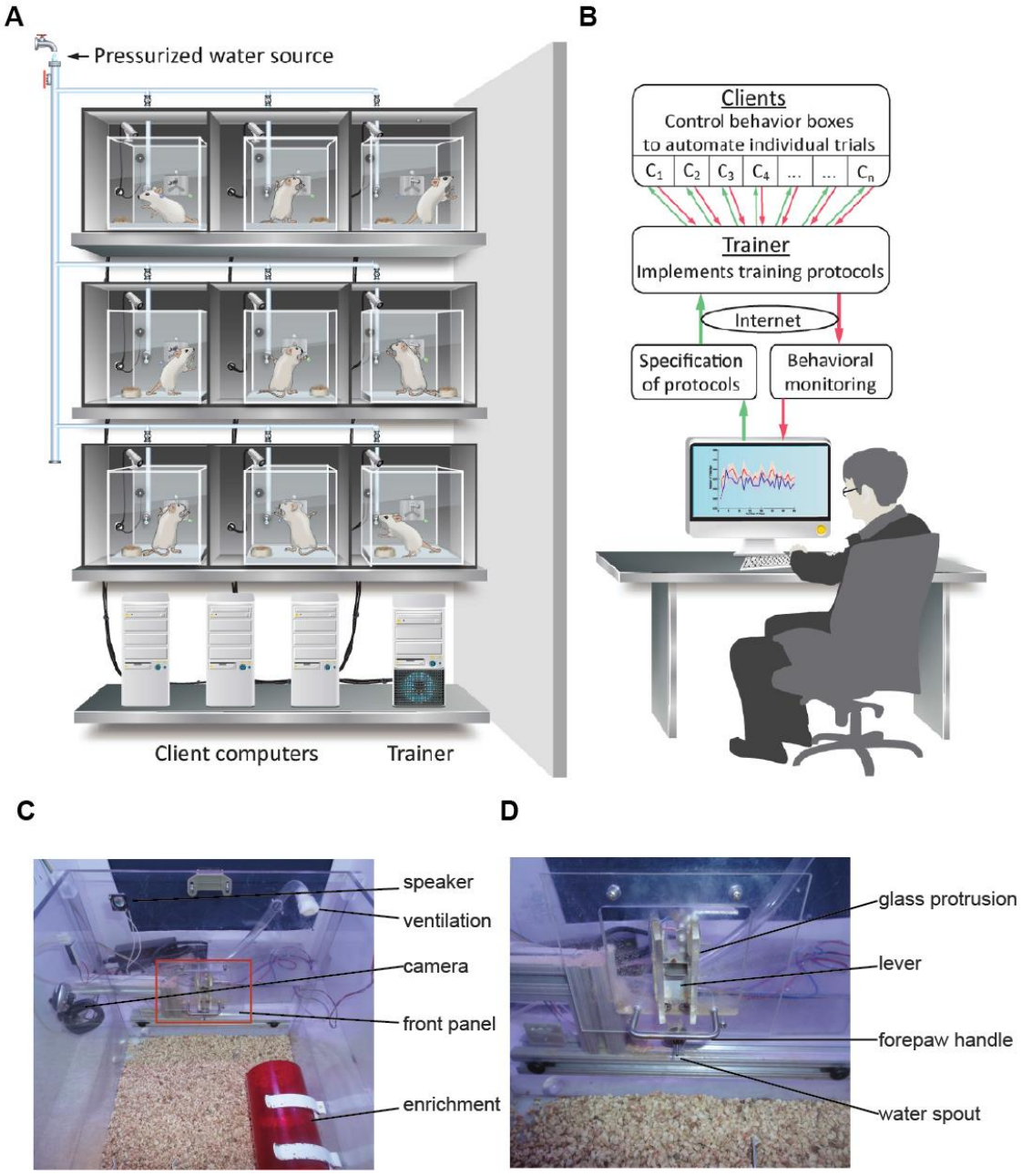

Figure 2.1 A fully automated training system for the two-press lever task.
Both (A) and (B) are adopted from. (A) Schematic of the hardware implementation. Custom-made training cages are directly controlled and managed by client computers with rules specified by the Trainer. Water is delivered though a computer-controlled solenoid valve connected to a central pressurized water source. (B) Logic of software implementation. Client computers control individual trials by directly interfacing to a behavior box. The Trainer monitors overall performance and implements the training protocol. The user controls and monitors training remotely over the internet. (C) Inner view of a training cage. The front panel in (D) is represented by the red rectangle. (D) Closer view of the front panel including the lever for the motor task.

Reward boundary updates (adopted from Kawai et al. 2015)

Since animals initially exhibited mean IPIs that were shorter than the target, we first adjusted the lower bound (LB) until they reached a median IPI greater than the target. At this point, the LB was set to the target. As performance improved, the upper bound (UB), which had been at 1100ms, was adjusted downward to increase the precision of the IPIs around the target.

Reward boundaries were updated based on the percentage of trials rewarded in the previous session (R). If R was between 30% and 40%, the boundaries remained unchanged. If R was below 30% and the median IPI below the target, the LB was lowered by I_1 = 10ms. If R was below 30% and the median IPI above the target, the UB was increased by I_1. If R was above 40% and the median IPI below target, the LB (in ms) was shifted to:

$$LB(t) = \min(\min(\max(Q_1, LB(t-1) + I_1), LB(t-1) + I_2), T)$$

where t = session number, Q_1 = 1st quartile, T = target IPI, I_2=50ms.

If R was above 40% and the median IPI above target and if

$$UB(t-1) - LB(t-1) > D$$

then, UB (in ms) was shifted to:

$$UB(t) = \max(\min(Q_3, UB(t-1) - I_1), UB(t-1) - I_2)$$

else

$$UB(t) = UB(t-1) - I_1$$

where Q_3 = 3rd quartile, D = 150ms.

To incentivize IPIs close to the target, rats received more water the closer their IPIs were to the target (Figure 2.2B). The distance between the target and each of the boundaries was divided into 5 equal size bins with the reward amount increasing in increments of 20 µl the closer the bin was to the target.

Lesion surgeries

Bilateral lesions were performed in two stages. The DLS or DMS was lesioned in the hemisphere contralateral to the paw used for the first lever press in motor sequences. If the performance recovered or remained unchanged after training resumed, the ipsilateral side was lesioned.

Animals, placed in a stereotaxic frame, were anesthetized with inhaled isoflurane. Craniotomy was performed over the targeted brain regions. The rate of injection of excitotoxin was equal to or slower than 0.1ul/min. DLS lesions (n=8) were made by injecting 0.7ul quinolinic acid (0.09M, Sigma-Aldrich), buffered to pH7.3 in 1X PBS, into four separate sites (0.175ul per site). The coordinates (in millimeters) (Paxinos and Watson 2007) relative to bregma (anteroposterior, mediolateral, dorsoventral), were: (-0.3, 3.6, -6.0), (-0.3, 3.6, -6.5), (+0.7, 3.6, -5.5) and (+0.7, 3.6, -6.0). For DMS lesions (n=8), the coordinates were (+1.2, 1.9, -4.5), (+1.2, 1.9, -5.5), (+0.2, 1.9, -4.5) and (+0.2, 1.9, -5.5). After injection, the pipette stayed in the injection site for 5 minutes to allow for drug diffusion. The pipette was then pulled out from the brain tissue slowly to prevent backflow of the drug. After surgery, animals were allowed to recover in the animal facility for 10 days before resuming behavioral training in their home cages.

Histology

After completion of the experiments, animals were anesthetized by intraperitoneal injection of 100mg/kg ketamine and 10mg/kg xylazine, and perfused transcardially with 1X PBS followed by a 4% buffered paraformaldehyde solution. The brains were sliced in 100µm coronal sections with a Vibratome and Nissl stained with crystal violet. Images of whole brain slices were taken by a VS120 Whole Slide Scanner (Olympus) under 10X.

Immunohistochemistry for the neuron-specific nuclear protein (NeuN) was performed in 2 DLS-lesioned animals and 2 DMS-lesioned animals for a second verification of the lesions. Specifically, after rinsing in 1X PBS, free-floating brain sections were incubated overnight at 4°C with a primary mouse anti-NeuN antibody (1:200, EMD Millipore MAB377) in 1X PBS containing 0.5% Triton X-100. After rinsing, the sections were incubated for 2 hours at room temperature with a secondary goat anti-mouse antibody (1:500, Alexa Fluor® 488 Goat Anti-Mouse IgG, Life Technologies) followed by another rinse. All sections were mounted with Fluoromount-G (SouthernBiotech) to reduce fluorochrome quenching.

Analysis of behavior and histology

Definition of Asymptotic Performance in Terms of Temporal Precision

We first estimated the coefficient of variation of the IPI distribution (CV) around each trial by calculating the CV over a 500-trial sliding window. We then fit a linear regression to the CV curve every 400 trials using a 2,700-trial window size. Asymptotic performance was defined as the point at which four consecutive linear regression fits had slopes less than 1.5×10^{-5} / trial.

Calculation of Performance Metrics

For Figure 2.2, the CV was calculated across 100 trials, and the moving average was then low pass-filtered with a 300-trial boxcar filter. The distance from the target was calculated similarly using the absolute deviation from the target as the variable. For Figure 2.3 (pre/post manipulation), we used the same procedure for learning but a smaller moving window (25 trials) and boxcar filter (50 trials). For Figures 2.5, 6 and 8, the first 1000 trials immediately after lesion were skipped in the analysis to account for non-specific effects of the lesions (see “mock break” in Figures 2.3 and 2.4). One out of 8 rats in the DLS group stopped working on the task after the contralateral lesion, and she was removed from the analysis.

Criterion Performance

Animals were deemed to have reached criterion performance when, for a 3,000-trial sliding window, the CV was less than 0.25 and the mean of the IPI distribution was within 10% of the target.

Lesion quantification

The lesioned area was estimated by inspecting brain sections with Photoshop. Lesioned regions were encircled under high magnification. The areas were automatically quantified by the software. For each animal, lesioned areas of 6 coronal brain sections spanning the striatum (Bregma: 1.56, 0.96, 0.48, -0.24, -0.72 and -0.96) were estimated. Lesioned areas between these sections were interpolated by a quadratic fit. The validity of such interpolation was verified in two rats. The total lesioned volume was calculated by integrating the estimated areas.

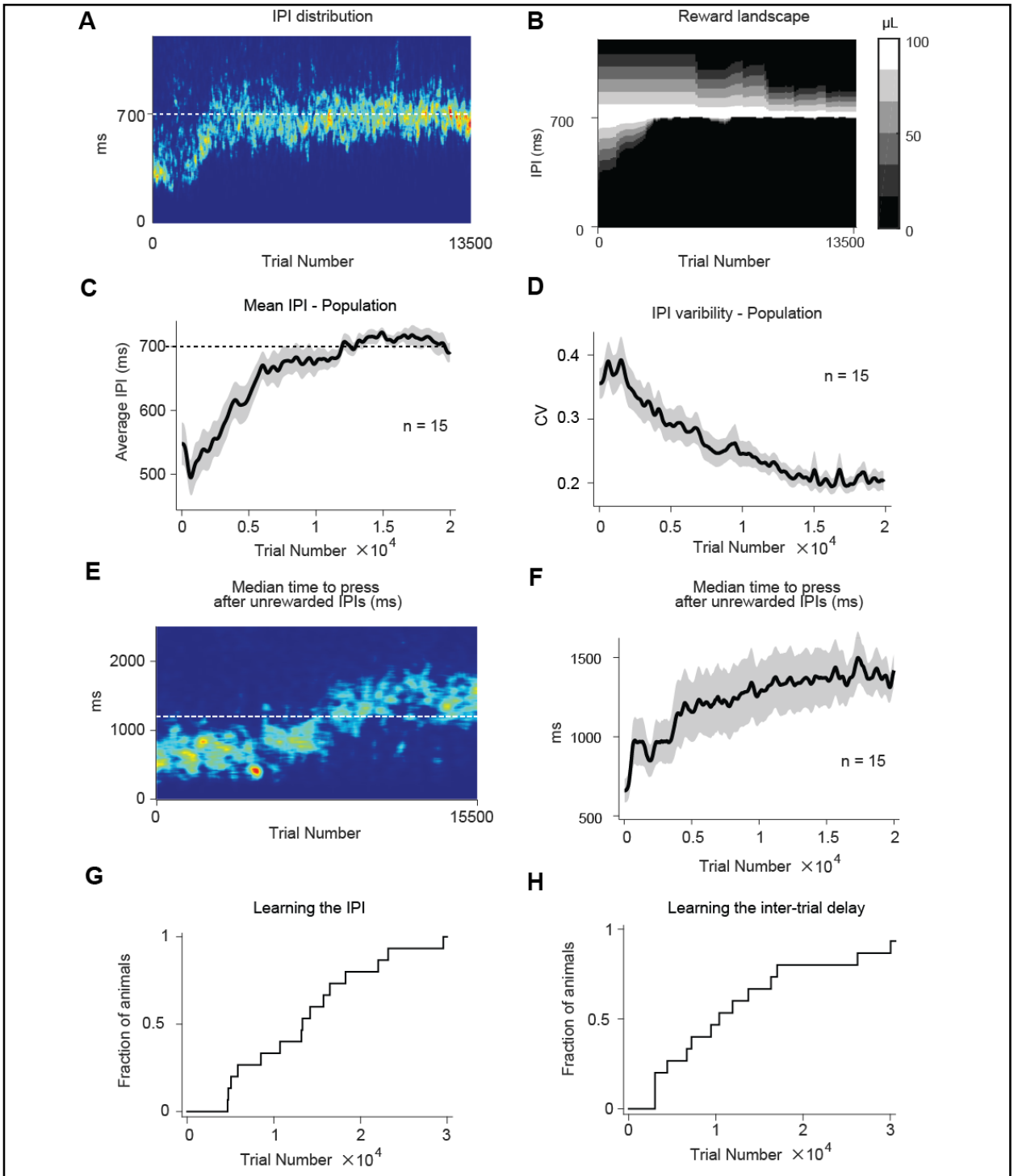

Figure 2.2 Rats trained in our novel paradigm learn to execute temporally stereotyped motor sequences.
(A) Density plot of the IPI distribution for a rat learning a 700-ms target IPI. (B) Graded reward landscape in (A). Amount of water rewarded was automatically adjusted based on IPI to ensure a reward rate of ~35%. (D and E) Learning curves showing the mean (D) and CV (E) of the IPI distribution as a function of training across all rats (n = 15). The shaded region denotes SEM. (E) Density plot showing the distribution of times between an unsuccessful second lever press and the subsequent press in the same rat as (A). (F) Learning curves of median delay after an unsuccessful second lever press (n = 15). The shaded area denotes SEM. (G and H) Cumulative histograms showing the fraction of animals that learned the task to criterion performance (see text) as a function of training.

Results

Learning the lever-pressing task

All 15 animals trained on the two-press task converged on 700-ms inter-press intervals (IPIs) before unilateral lesion of the DMS (n = 8) or DLS (n = 7). In early training, IPIs were short (507ms ± 103ms, first 1000 trials) and highly variable (0.39 ± 0.10 CV, first 1000 trials) on average. When training progressed and the reward range of the IPI gradually narrowed to around 700ms (Figure 2.2B, see method), the mean IPI also shifted toward the target (Figures 2.2A and 2.2C) and the variation decreased (Figures 2.2A and 2.2D). On average it took 13,696 trials ± 7,457 trials to reach asymptotic performance, defined as the mean IPI within 10% of the target and the CV of IPI being less than 0.25. All animals eventually passed the criteria (Figure 2.2G). The asymptotic mean IPI before lesion was 704ms ± 24ms with a CV of 0.18 ± 0.03. Furthermore, the emerged movement patterns became highly stereotyped, with each animal converging on a unique paw trajectory (Kawai et al. 2015, or see Chapter 1).

Although not explicitly required in the task, all rats learned to withhold pressing the lever for a predetermined 1.2s after a non-rewarded trial (Figure 2.2H), thus allowing the next trial to be quickly initiated after a failed (unrewarded) IPI. This makes training more productive. The median time to the next lever press after unrewarded IPIs exceeded 1.2s after, on average, 15,716 ± 1,793 trials (Figures 2.2E and 2.2F).

DLS but not DMS is required for executing the learned motor sequences

To probe whether the striatum is required for the execution of the complex motor sequences we train, we lesioned either the DLS (Figure 2.3A) or DMS (Figure 2.3B) after the animals reached asymptotic performance. Lesions were first performed on the hemisphere contralateral to the forepaw used for the first lever press in a sequence. The region and size of lesions (Figure 2.7) were comparable to previous

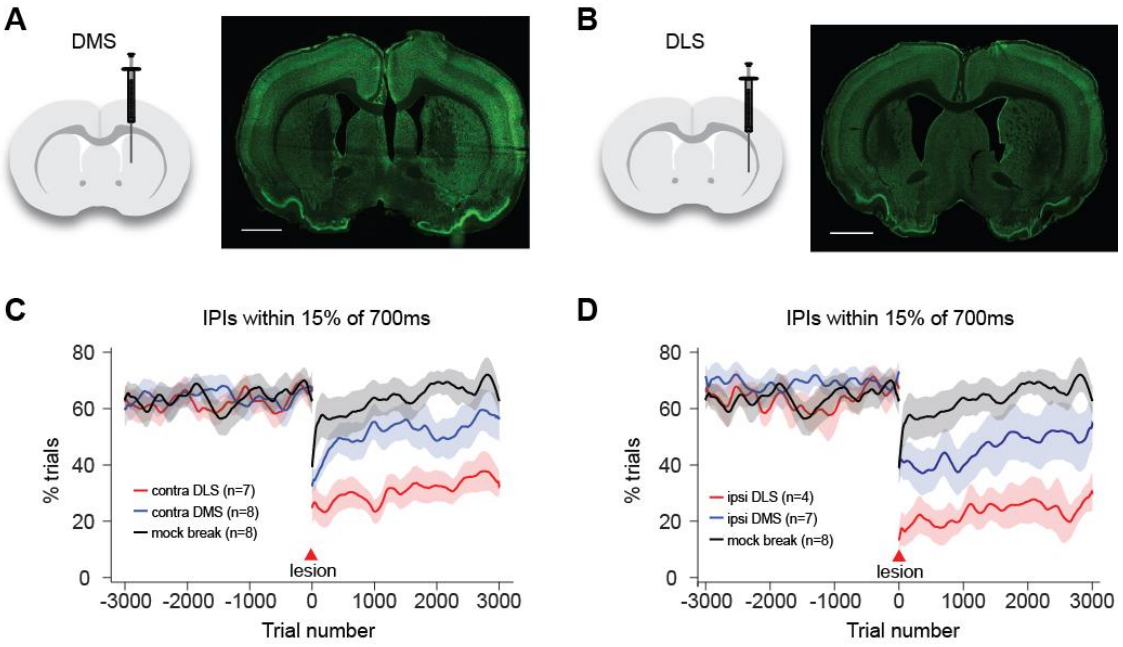

Figure 2.3 The DLS is required for execution of learned motor sequences while the DMS is required to a lesser extent. (A and B) DLS (A) and DMS(B) lesions. Left: Schematics from coronal slice (Bregma: +0.7, Paxinos and Watson 2007) showing the mediolateral position for injection of quinolinic acid (1.9 for the DMS and 3.6 for the DLS). Right: Fluorescent images of NeuN immunohistology on corresponding coronal slices from bilaterally lesioned brains. NeuN is expressed in neurons but not glials. The darker regions within the dorsal striatum represent lesioned tissues. The scale bar represents 2mm. (C and D) Percentage of trials with IPI within 15% of 700ms before and after contralateral (C) and ipsilateral (D) lesions. All manipulations were done after the animals had reached asymptotic performance on the task. The shaded regions denote SEM across animals.

studies (Castañé, Theobald, and Robbins 2010; Featherstone and McDonald 2004; Yin, Knowlton, and Balleine 2004). Tissue in the ventral striatum and the overlying cortex were spared. Spontaneous rotating behavior was occasionally observed on the day after surgery, but resolved within 2-3 days. After 10 days of recovery, no major non-specific deficits in motor control were observed, and behavioral training resumed.

If the basal ganglia are involved in storing and executing the learned motor sequences we train, significant deficits in performance and alteration in kinematics would be expected after striatal lesions. Specifically, if the subcortical sensorimotor loop (see Chapter 1 for an introduction to subcortical loops) is required for motor skill execution, performance would be expected to degrade after the lesion of DLS,

which is part of the sensorimotor loop. Likewise, if the associative loop is essential for the task, performance would be significantly affected after DMS lesions.

We found that contralateral DLS lesions severely degraded task performance. In contrast, animals with contralateral DMS lesions performed similarly to pre-lesion. In the DLS group (n = 7), the percentage of trials with IPI falling within 15% of 700ms (or 595ms - 805ms) dropped from 62% ± 7% before lesion to 31% ± 12% after lesion (first 3000 trials, p = 0.0028, Figure 2.3C), comparable to the performance after 2 weeks of training from the beginning. On the other hand, in the DMS group (n = 8), the same indicator decreased from 64% ± 11% to 51% ± 16% (p = 0.016), worse than the animals with 10-day mock breaks by 12% (p = 0.022), but better than the DLS group by 20% (p = 0.015, Figure 2.3D).

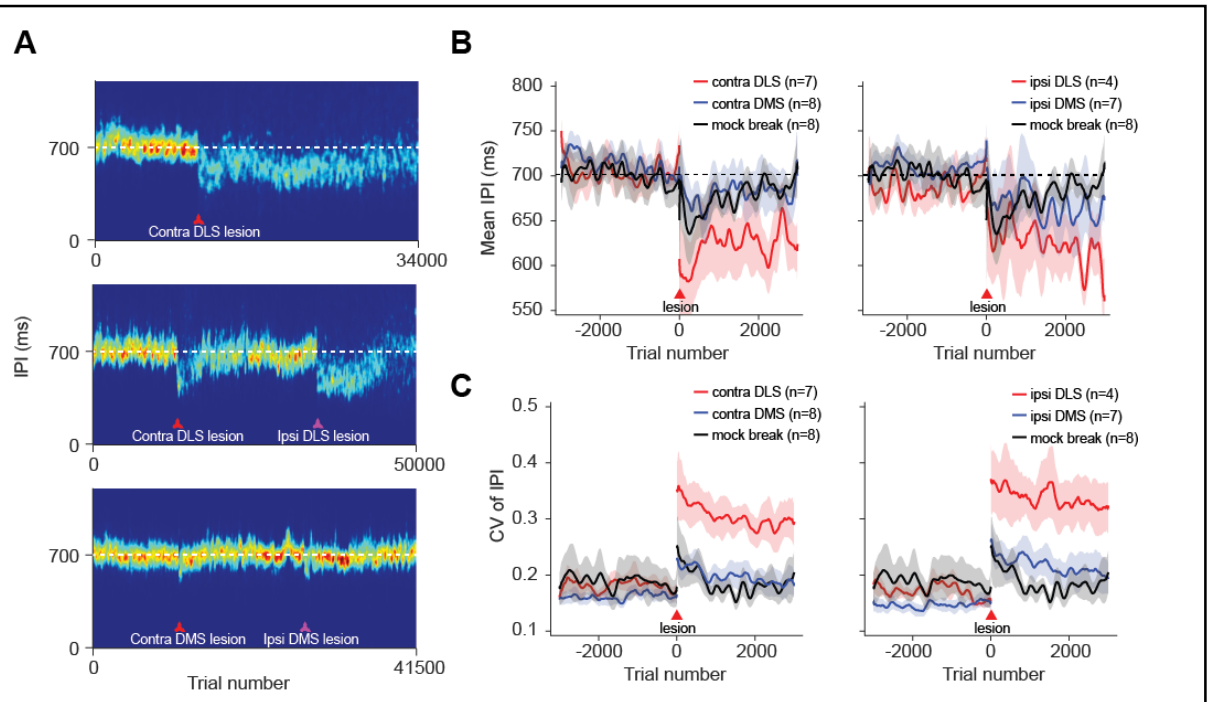

Figure 2.4 Temporal precision of motor sequences was disrupted by DLS but not DMS lesions.
(A) Density plot of the IPI distribution before and after lesions. Top: Contralateral DLS lesion. Middle: Two-stage bilateral DLS lesions. Bottom: Two-stage bilateral DMS lesions. (B) Mean IPI of the population before and after contralateral (left) and ipsilateral (right) lesions. (C) Mean CV of IPI of the population before and after contralateral (left) and ipsilateral (right) lesions. All contralateral lesions were done after the animals had reached asymptotic performance on the task. Ipsilateral lesions were done only when the performance recovered or remained intact after contralateral lesions. The shaded regions denote SEM across animals.

The performance degradation in the DLS group was associated with changes in both the deviation of mean IPI from the 700-ms target and an increase in the CV of the IPI distribution. In the DLS group, mean IPI decreased from 704ms ± 28ms to 626ms ± 72ms after lesion (first 3000 trials, $p = 0.15$, Figures 2.4A top and 2.4B), and the CV of the IPI distribution increased from 0.18 ± 0.03 to 0.29 ± 0.09 ($p = 0.048$, Figures 2.4A bottom and 4C). Conversely, both indicators in the DMS group were comparable to the control group. Mean IPI changed from 703ms ± 22ms to 684ms ± 66ms in DMS animals ($p = 0.64$) and from 693ms ± 36ms to 690ms ± 23ms in control animals ($p = 0.78$). The CV changed from 0.17 ± 0.03 to 0.19 ± 0.06 ($p = 0.24$) in the DMS group and from 0.19 ± 0.08 to 0.19 ± 0.06 in the control group ($p = 0.21$).

In the DLS group, the delay time to next lever press after unrewarded IPIs was also affected. Four out of 6 animals that learned the predetermined 1.2s inter-trial delay were not able to keep the median delay above 1.2s after lesioning. The percentage of delay time above 1.2s across animals decreased from 71.7% ± 7.6% to 41.0% ± 8.2% ($p = 0.03$ in paired t-tests, 500 trials, Figures 2.5A top and 2.5B) accompanying a drop of two-press trials from 80.9% ± 4.5% to 45.6% ± 7.0% ($p = 0.001$, 500 trials, Figure 2.5C). In contrast, all of the 7 DMS animals that learned the predetermined 1.2-s delay retained it after lesion. The change in percentage of delay times above 1.2s was not significant (from 77.0% ± 5.7% to 72.4% ± 6.6%, $p = 0.54$, Figures 2.5A bottom and 2.5B), and the drop of two-press trials was far less severe (from 81.3% ± 3.6% to 70.3% ± 4.8%, $p = 0.04$, Figures 2.5C).

To visualize the change of task-related movement patterns, we examined how long the lever was depressed for the first press, relative to how long it took to press it the second time (Figures 2.6). Before lesion, the distribution of both durations was dense, reflecting the stereotypy in movement kinematics (Figures 2.6A and 2.6B). After DLS but not DMS lesions, the distribution shifted and became more diffuse, reflecting increased variability in movement patterns and kinematics. In the DLS group, the centroid shifted by 0.20 ± 0.07 in the parameter space after lesion, higher than the DMS (0.09 ± 0.04, $p = 0.20$) and control groups (0.09 ± 0.02, $p = 0.15$) (Figure 2.6C). The average pairwise distance, measuring cluster

dissimilarity, between the pre- and post-lesion distributions was 0.39 ± 0.05in the DLS animals, larger than the DMS (0.24 ± 0.03, p = 0.032) and control groups (0.27 ± 0.03, p = 0.066) (Figure 2.6D). The result suggests that the DLS, but not the DMS is important for maintaining the learned movement patterns.

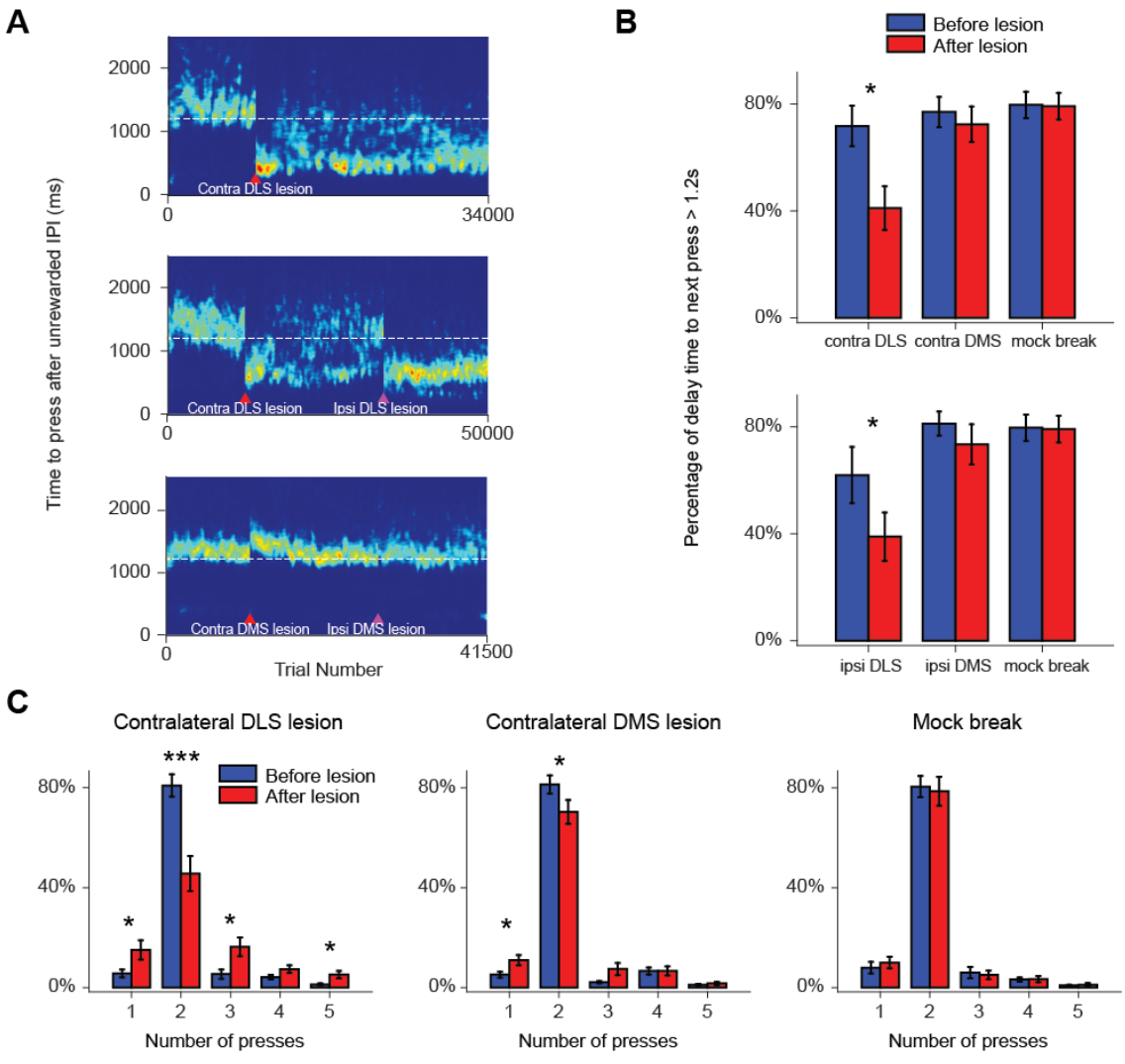

Figure 2.5 Learned structure of inter-trial delay was disrupted by DLS lesions.
(A) Density plot of the delay to next lever press before and after lesions. Top: Contralateral DLS lesion. Middle: Two-stage bilateral DLS lesions. Bottom: Two-stage bilateral DMS lesions. (B) Percentage of delay time to next press after unrewarded IPIs longer than the predetermined 1.2s (in 500 trials) before and after contralateral (top) and ipsilateral (bottom) lesions. (C) Percentage of trials with 1 to 5 lever presses (in 500 trials) before and after manipulations. All contralateral lesions were done after the animals had reached asymptotic performance on the task. Ipsilateral lesions were done only when the performance recovered or remained intact after contralateral lesions. Sample numbers are the same as in Figure 2.4. In paired tests, '*' and '***' represent $p < 0.05$ and $p < 0.001$, respectively. Error bars denote SEM across animals.

A fraction of animals with unilateral DLS lesions recovered after the lesion, but it took longer than the DMS animals, as quantified below. Performance was considered to have recovered if it fulfilled the asymptotic performance criteria, defined as the mean IPI within 10% of the target and the CV of IPI being less than 0.25. Within 4 weeks (or 84 one-hour training sessions), 5 out of 8 DLS animals and all of the

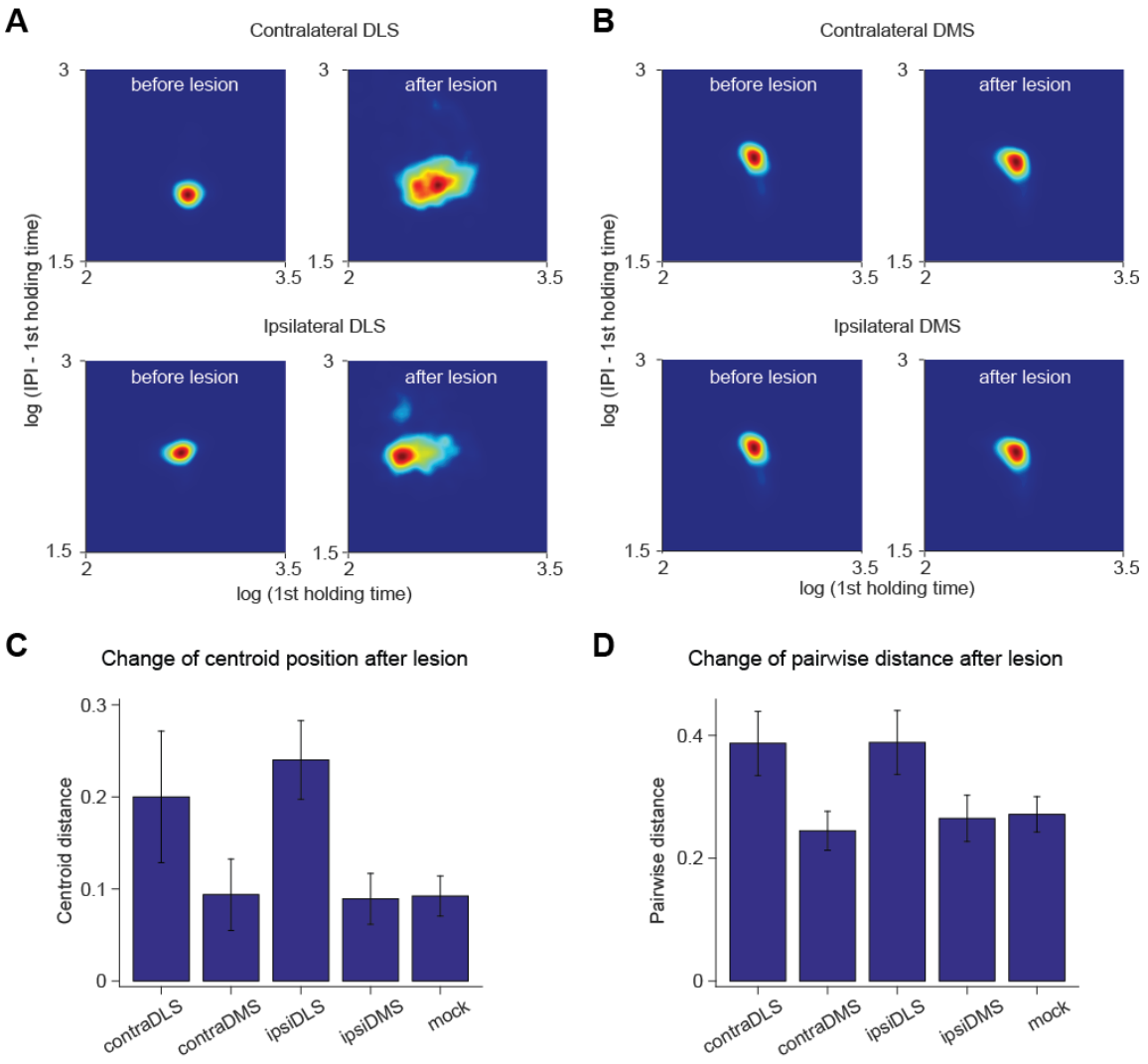

Figure 2.6 Learned movement patterns was disrupted by DLS but not DMS lesions.
(A and B) Density plot of the logarithm of the two parameters, 1) the holding time of the first lever-press (1st holding time) and 2) the time between the first lever-release and the second lever-press (IPI – 1st holding time) before and after lesions in a DLS rat (A) and a DMS rat (B). Each plot consists of 2000 trials. (C) Bar chart showing the population mean of the distances between the two centroids of the clusters plotted above before and after manipulations in 500 trials. (D) Bar chart showing the population mean of the pairwise distances between the two clusters plotted above before and after manipulations in 500 trials. Sample numbers are the same as those in Figures 2.4 and 2.5. Error bars represent SEM.

DMS and control animals recovered. On average, the DLS animals that recovered did so in 7028 ± 3821 trials, about twice as long as DMS animals (3888 ± 1438 trials, $p = 0.056$) and control animals (3413 ± 197 trials, $p = 0.029$).

Since the basal ganglia project to downstream motor areas bilaterally (Deniau et al. 1977; Takada et al. 1994; Parent, Lévesque, and Parent 1999), the ipsilateral striatum may also be involved in our motor task, particularly in the rats that recovered from lesion. To address the issue, we lesioned the ipsilateral DLS in 4 of the 5 recovered DLS animals. These lesions led to another significant performance degradation (Figure 2.3D). Multiple indicators including the mean IPI (Figures 2.4B), the CV of IPI (Figures 2.4C), the delay time to lever press after unrewarded IPIs (Figures 2.5B), and the movement patterns (Figures 2.6) were all affected as in contralateral DLS lesions, implicating the role of ipsilateral DLS in the execution of learned sequences. Similarly, we lesioned the ipsilateral DMS in 7 out of 8 recovered DMS animals. In contrast to the DLS group, the same performance metrics (Figures 2.4-2.6) were not much affected by ipsilateral DMS lesion.

These results reveal that the basal ganglia are essential for storing and executing learned motor sequences. In terms of the striatum, we found that the DLS is far more important than the DMS. Animals with bilateral DMS lesions could execute the task as well as before, but this was not the case for DLS-lesioned animals. Given that the motor cortex is not involved in execution but learning of the motor skill (Kawai et al. 2015), and that the DLS receives strong motor cortical input (McGeorge and Faull 1989; Voorn et al. 2004; Haber 2003), we propose that the basal ganglia, and possibly the subcortical sensorimotor loop, are “tutored” by the motor cortex via its projections to the DLS during motor sequence learning.

Correlation of DLS lesion volume with performance degradation

The DLS lesions likely did not cover the entire striatal region with motor cortex efferents, which may explain the partial recoveries we observed and the difference in deficits across animals. If the DLS is essential for skill execution we would expect a correlation between lesion volume and performance degradation.

To test this, lesion volume was quantified in the photomicrographs of the Nissl-stained brain slices (Figure 2.7A). Under 5X magnification, the border between the lesioned and intact tissues could be delineated by observation (Figure 2.7A top). Under 20X magnification, cell bodies of neurons with diameters over 10μm, could be found easily in intact tissue (Fig. 2.7A bottom right). In contrast, mainly glial cells could be observed in lesioned tissue, with a near complete loss of neuronal cell bodies (Fig. 2.7A bottom left). The pharmacological lesions of the DMS (Figure 2.7B) and the DLS (Figure 2.7C) were comparable to previous studies (Castañé, Theobald, and Robbins 2010; Featherstone and McDonald 2004; Yin, Knowlton, and Balleine 2004), with DMS lesions located more anteriorly and medially, and DLS lesions more posteriorly and laterally. More importantly, DLS lesions in our animals mostly overlapped with the major striatal region receiving motor cortical projections as revealed by fluorescent anterograde tracing (Figure 2.7D, adopted from the mouse connectivity database of the Allen Brain Institute) (Oh et al. 2014).

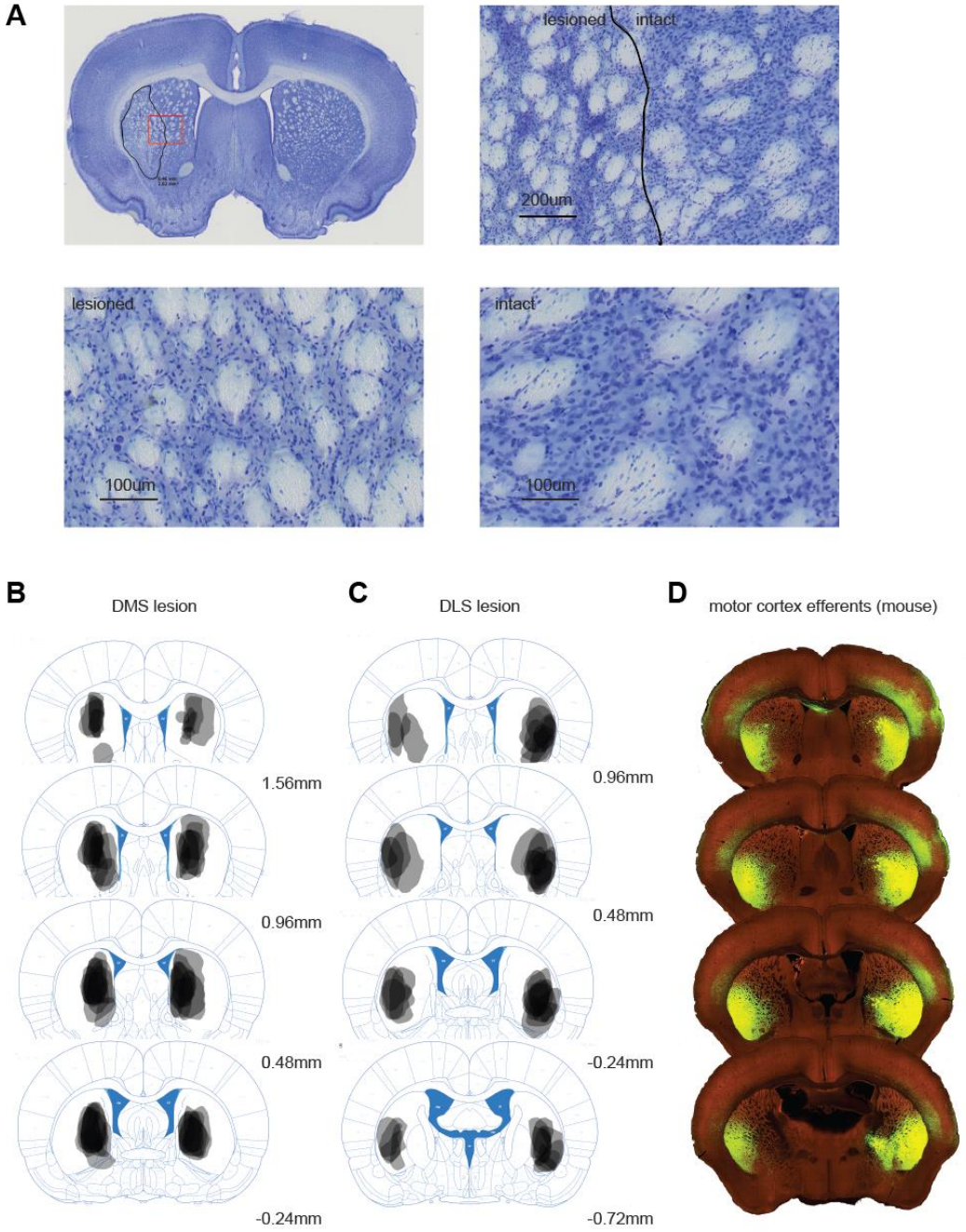

Figure 2.7 Histology of DLS and DMS lesions. (A) Photomicrographs of the coronal nissl-stained slices from a unilateral DLS lesioned rat. Top left: The lesioned region, as indicated by the black border, showed shrinkage of brain tissue and lighter color of staining when compared with the intact side. Top right: Part of the lesion-intact boundary in (A, the red rectangle) magnified. Bottom left: Under 20X, the lesioned region showed mostly glial cells, with nearly complete loss of neurons. Bottom right: Under 20X, the intact region showed dense cell bodies with diameters over 10μm. (B and C) Schematics of a series of coronal sections illustrating the extent of DMS (B) and DLS (C) lesions. The level of greyness represents coincidence of lesions from different animals. The diagrams are based on a rat brain atlas (Paxinos and Watson, 2007). The numbers indicate distance in mm from bregma. (D) Fluorescent imaging of AAV anterograde tracing from M1 (and partially M2) to the striatum in mice. Coronal sections correspond to those in (C). (D) was adopted from the connectivity database of Allen Brain Institute: http://connectivity.brain-map.org/projection/experiment/180719293.

The lesion areas in at least 6 brain sections spanning the striatum were measured for lesion volume estimation (see Methods). Average lesion volumes of the DMS and DLS groups were comparable (6.06 ± 0.61 mm^3 for the DMS and 5.79 ± 0.72 mm^3 for the DLS, p = 0.78 in the t-test, Figure 2.8A). Interestingly, there were significant correlations between the DLS lesion volume and multiple performance metrics, including the percentage of IPIs within 15% of target (r = -0.76, p = 0.01, Figure 2.8B), the deviation in mean IPI (r = 0.61, p = 0.05, Figure 2.8C), the change in CV (r = 0.60, p = 0.05, Figure 2.8D) and the percentage of delay time to next lever-press after unrewarded IPIs above the predetermined 1.2s (r=-0.69, p=0.02, Figure 2.8F). In contrast, DMS lesion size was not correlated with either of the five performance metrics in Figure 2.8. The results suggest stronger deficits in performance could be caused by removing a larger proportion of DLS neurons. Such performance deficits were specific to learned sequences since no gross motor deficits were observed 10 days after lesion by quinolinic acid (see also Yin 2010; Castañé, Theobald, and Robbins 2010; Whishaw et al. 2007).

Finally, we found that DLS lesion size was correlated with the recovery of IPIs within 15% of target. Recovery is defined as the improvement 10,000 trials after lesion divided by the degradation caused by lesion. With larger lesions, recovery was poorer (r = -0.78, p = 0.01, Figure 2.8G). The result provided motivation to address whether DLS is also involved in learning the motor skill (Chapter 4).

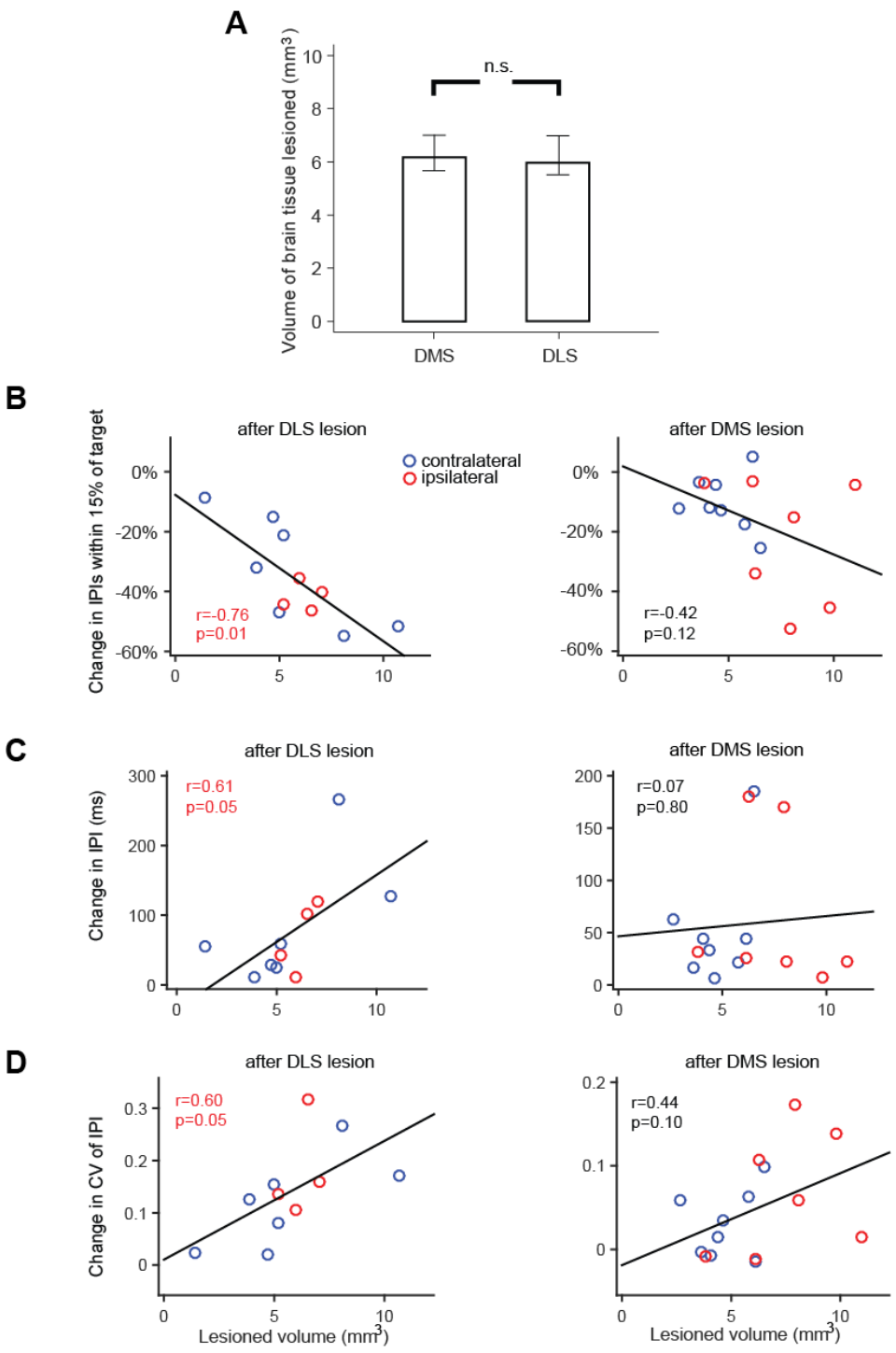

Figure 2.8 Correlation of DLS lesion volume with performance degradation. (A) The mean lesioned volumes of DMS (15 lesions from 8 animals) and DLS groups (11 lesions from 7 animals) were comparable. Error bars represent SEM. (B to G) Correlation plots of multiple performance parameters versus lesioned volume. Parameters include the change in trials with IPIs within 15% of the 700-ms target (B), the surge in mean IPI (C), the increase in CV of IPI (D), the average pairwise distances of the 2 clusters in the parameter space of Figure 6 before and after lesions (E), and the percentage of time to next lever press after unrewarded IPIs below 1.2s (F). 1,000 trials before and after lesions were considered in each sample point. (Description continues next page.)

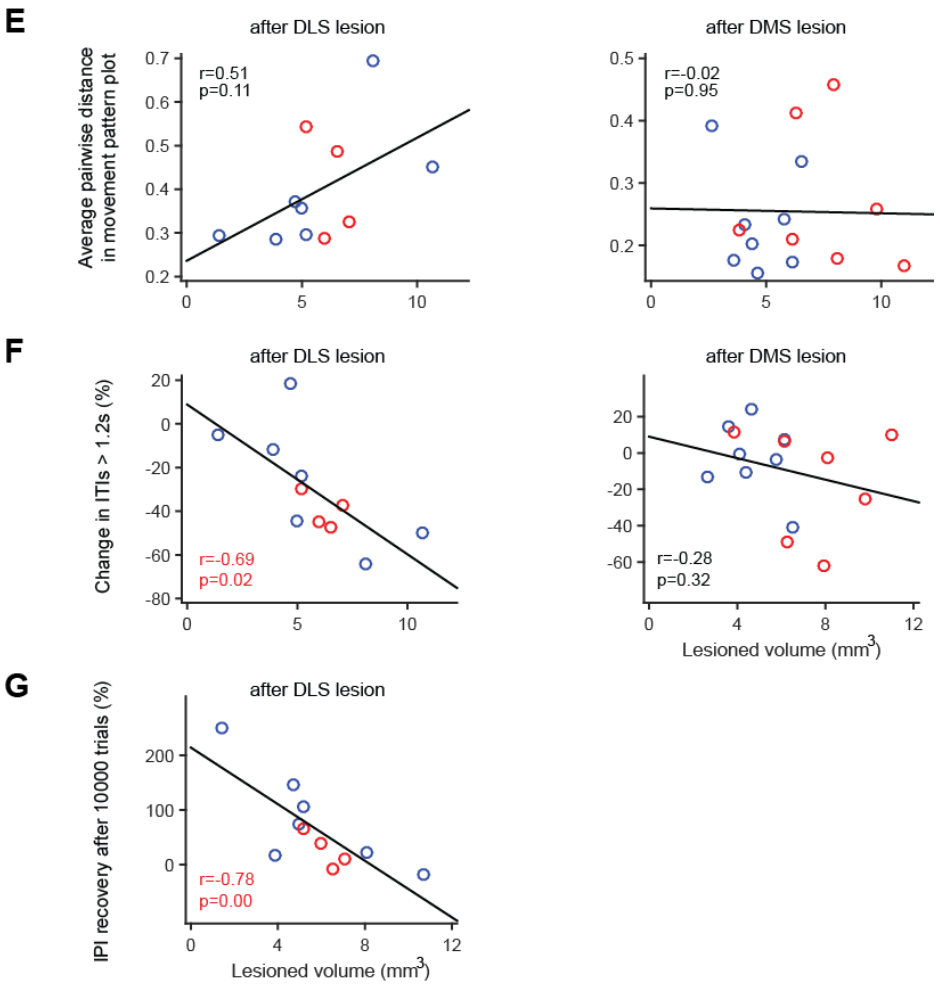

Figure 2.8 (continued). Correlation of DLS lesion volume with performance degradation. (G) Correlation plot of recovery of IPIs within 15% of target 10,000 trials after lesion. Recovery is defined as the improvement 10,000 trials after lesion divided by the degradation caused by the lesion; therefore, recovery can exceed 100%. Computing window is 1,000 trials. Pearson correlations with statistical significance ($p<=0.05$ in non-directional tests) are denoted in red.

Discussion

To examine the role of the basal ganglia in the execution of learned motor sequences, we trained rats to perform spatiotemporally precise lever-press sequences using a novel motor skill paradigm we developed in the lab (Kawai et al. 2015, Figures 2.1 and 2.2). DLS lesions disrupted motor performance (Figure 2.3) in terms of timing (Figure 2.4), trial structure (Figure 2.5) and movement patterns (Figure 2.6). Furthermore, performance degradation was correlated with the volume of the DLS lesions (Figure 2.8).

DMS, on the other hand, is largely dispensable for generating the learned motor sequences (Figures 2.3-2.6). The results implicate that the basal ganglia are the subcortical substrate for storing and generating the motor cortex-independent motor sequences we train, and that the sensorimotor arm of the basal ganglia, as revealed by DLS lesions, is crucial for the sequences' execution.

Basal ganglia as subcortical substrates for execution of cortex-independent sequences

The basal ganglia are known to be involved in learned motor skills (Graybiel et al. 1994), but previous studies often assumed that the functionality of basal ganglia circuits is mediated through the motor cortex (Hikosaka et al. 2002; Doyon et al. 2009) via the cortico-basal ganglia-thalamic loop (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995). However, our results suggest that the basal ganglia can contribute to motor skill execution also through its projections to subcortical motor controllers (Kawai et al. 2015).

The basal ganglia do not have direct access to motor neurons, and hence must exert their effects by modulating subcortical controllers. The targets of the basal ganglia's output (GPi/SNr) include the superior colliculus (Krout et al. 2001; Krout, Belzer, and Loewy 2002), pedunculopontine nucleus (Erro, Lanciego, and Giménez-Amaya 1999) and reticular nuclei (Krout, Belzer, and Loewy 2002). Our results suggest that the basal ganglia coordinate and organize subcortically generated movements into smooth and efficient task-specific motor sequences. Thus our results implicate that subcortical motor nuclei, which are widely presumed to encode innate movement patterns (von Euler 1983; Gray et al. 2001; Lund 1991; Rossignol 2010; Grillner 2011), can be recruited by the basal ganglia to generate complex sequences of movements. Our results suggest that the canonical view of cortical/subcortical roles in control of learned behaviors should be revisited.

A tutor function for the motor cortex – DLS projections

Our lab has shown that the motor cortex is not required for the execution of the non-dexterous motor sequences we train (Kawai et al. 2015). Rats with motor cortex lesions, however, were not able to learn the task. This raises the possibility that the motor cortex "tutors" subcortical motor controllers during learning, as it can directly influence brainstem central pattern generators and the basal ganglia through corticobulbar tracts (Kuypers 1958) and corticostriatal projections (Shepherd 2013; Doig, Moss, and Bolam 2010). By adapting and reorganizing subcortical motor networks, the motor cortex can contribute to the acquisition of complex task-specific motor sequences, without being a necessary controller for the learned behavior. Our results suggest that the DLS may be the target of the motor cortex's tutoring.

Comparisons with prior striatal studies on motor sequence execution

Our results are consistent with previous studies implicating the DLS in the execution of various motor sequences, including innate (Berridge and Fentress 1987; Pellis et al. 1993), externally cued (Miyachi et al. 1997; Matsumoto et al. 1999; Robbins 2002; Bailey and Mair 2006) and self-initiated sequences (Yin 2010). However, it is important to point out the differences in these studies. While innate behaviors offer an excellent model of stereotyped complex motor sequences, they do not require training that potentially recruits different neural substrates (Grillner and Wallén 2004). On the other hand, externally cued sequences such as the popular serial reaction time task (Robbins 2002; Bailey and Mair 2006) require learning, but they are confounded by the requirement of following sensory cues, making it difficult to distinguish whether lesion-induced deficits are due to an inability to perform learned motor sequences or an inability to follow cues. Another major distinction is that our skills are verifiably motor cortex-independent, and hence our findings implicate the subcortical basal ganglia loop, whereas previous studies could not make this distinction. Thus, just as the basal ganglia are thought to modulate cortical controllers, we show that they critically modulate subcortical ones as well.

Potential roles of the DMS in skill execution

Although my lesion experiments show that the DMS is largely dispensable for executing motor sequences, it does not imply that the DMS is not involved in the neural representation of the movements. Neuronal activity in the DMS has been found to correlate with location, direction and movement in a self-initiated navigation task (Wiener 1993). Neurons that encode start/stop signals and the entire motor sequence have also been recorded in both the DLS and the DMS (Jin and Costa 2010; Jin, Tecuapetla, and Costa 2014). Furthermore, as opposed to self-initiated sequences, the DMS possibly plays an important role in externally guided movements in which integration of information from the prefrontal cortex and visual/auditory cortices is necessary during early stages (Miyachi et al. 1997; Matsumoto et al. 1999). Likewise, the DMS could be important for early learning in our task, a question we address in Chapter 4.

Remaining questions and follow-up experiments

Several questions remain after our striatal lesion study. First, can we rule out the possibility that performance deficits were caused by lesion-induced "aberrant activity" spreading to downstream motor circuits (Ayalon et al. 2004; Shin, Aparicio, and Ivry 2005) rather than loss of striatal function? Since pallidotomy can block aberrant firing patterns from the basal ganglia (Mink 1996; Vitek and Giroux 2000; Okun and Vitek 2004), we decided to lesion the GPi (Chapter 3). Second, if the DMS is not required for execution of motor sequences, is it involved in other functions such as acquisition of motor sequences? To answer this question, we bilaterally lesioned the DMS and the DLS before training the motor task and examined learning outcomes in the two groups (Chapter 4). Finally, if the DLS is required for execution of the learned motor task, how exactly does it contribute at the neuronal level? My colleagues are, hopefully, untangling the mystery by performing tetrode recording in the DLS and searching for neural correlates for various motor parameters in our task.

Summary

We trained animals to perform complex learned motor sequences with a novel paradigm. Lesions of the DLS significantly disrupted performance. In contrast, the DMS is largely dispensable for executing the motor skill. We suggest that the sensorimotor basal ganglia, possibly “tutored” by the motor cortex, are essential for executing cortex-independent motor sequences.

Chapter 3 Role of the GPi in motor sequence execution

Introduction

In Chapter 2, I examined the role of the striatum—the major input nucleus of the basal ganglia—in the execution of learned motor sequences. In this chapter, I will describe the role of the GPi—one of the basal ganglia output nuclei—in the same motor skill that I previously described.

One confound of striatal lesions is that dysfunctions of the striatum might induce aberrant neural activity, which in turn could disrupt neural activity in downstream motor areas including various motor controllers. Motor deficits associated with various basal ganglia diseases that affect striatal function, such as Parkinson's and Huntington's diseases, have been accounted for in these terms (Hammond, Bergman, and Brown 2007; Jenkinson and Brown 2011; Marreiros et al. 2013; Uhlhaas and Singer 2006; Mink 1996). Pathological neural dynamics in the striatum could disrupt the normal neural activities and functions of the basal ganglia-recipient regions like the brainstem and motor cortex, leading to the deficits in skill execution I described in Chapter 2. Hence, those deficits may not reflect loss-of-function in the basal ganglia (Ayalon et al. 2004; Shin, Aparicio, and Ivry 2005; Desmurget and Turner 2010; Turner and Desmurget 2010).

Clinically, pallidotomy (or GPi lesioning) has been performed to relieve symptoms of Parkinson's disease. In humans, these interventions largely disconnect the basal ganglia from the cortex, brainstem and thalamocortical structures, preventing aberrant activity from interrupting downstream motor controllers (Mink 1996; Vitek and Giroux 2000; Okun and Vitek 2004). To rule out that the effects I described in Chapter 2 are due to aberrant striatal activity, we will lesion the GPi, thereby blocking one of the output streams of the basal ganglia and further probing the role of the basal ganglia circuit in skill execution. If the effect of our DLS lesions reflects aberrant output, GPi lesions should not have much effect.

However, significant deficits in GPi-lesioned animals would suggest that the basal ganglia makes essential contributions to the execution of the skills we train.

The basal ganglia have multiple output nuclei, the major ones being the GPi and SNr. We chose the GPi over the SNr in our lesion study because: (1) Excitotoxic lesions of the GPi, which is found within a sea of axons in the internal capsule (Paxinos and Watson 2007), are cleaner as they can kill GPi neurons without affecting other structures of fiber pathways. Lesions of the SNr, on the other hand, will unavoidably damage the SNc, which is adjacent to the SNr (Paxinos and Watson 2007) and important for reinforcement learning (Samson, Frank, and Fellous 2010; Dayan and Balleine 2002). (2) Lesions of the GPi, but rarely the SNr, are used to treat Parkinson's disease (Obeso et al. 2009; Lozano et al. 1995; Baron et al. 1996), implicating the effectiveness of pallidotomy in blocking striatal aberrant signals. (3) The SNr is involved in movements of the eyes, head, and neck; whereas the GPi is related mainly to axial and limb movements (Gerfen and Bolam 2010), and hence more relevant to our lever-press task.

In contrast to the striatal manipulations discussed in Chapter 2, previous animal studies do not indicate important roles for the GPi in motor skills. The Schwabe lab has shown that GPi lesions do not affect performance in a rotating rod task, a test for the coordination of limbs (Lütjens, Krauss, and Schwabe 2011). Also, Desmurget and Turner reveal that GPi inactivation in trained monkeys does not impair learned movement sequences in a visuomotor task (Desmurget and Turner 2010), suggesting that the basal ganglia may not be required for explicitly learned motor sequences. However, whether the basal ganglia are required for motor sequences learned without instructional cues has not yet been resolved.

In the present study, we trained rats to perform the lever-press task described in Chapter 2 and pharmacologically lesioned the GPi to examine the role of the basal ganglia in executing learned motor sequences. We find that GPi lesions acutely disrupt performance, with roughly half of the animals recovering after re-training.

Method

Behavioral training

The behavioral task is the same as described in Chapter 2. See Methods in Chapter 2 for details of the training procedure, behavioral analysis, and histology.

Lesion surgeries

Bilateral lesions were performed in two stages. The GPi was lesioned in the hemisphere contralateral to the paw used for the first lever press in motor sequences. If the performance recovered or remained unchanged after training resumed, the ipsilateral side would be lesioned.

Animals were anesthetized with inhaled isoflurane on a stereotaxic frame. Craniotomy was performed over the targeted brain region. The rate of injection of the excitotoxin was equal to or slower than 0.1ul/min. GPi lesions were made by injecting 400nL of 1% ibotenic acid in one single site. The coordinates in millimeters (Paxinos and Watson 2007), relative to bregma (anteroposterior, mediolateral, dorsoventral), were: (-2.3, 2.8, -7.5). After injection, the pipette stayed in the injection site for five minutes to allow for drug diffusion. The pipette was then pulled out from the brain tissue slowly to prevent drug backflow. After surgery, animals were allowed to recover in the animal facility for 10 days before resuming behavioral training in their home cages.

Histology revealed that the injection of 400nL ibotenic acid might damage part of the brain nuclei adjacent to the GPi. To minimize unspecific damage, in 8 animals, the location of the GPi was estimated by single electrode recording to improve the accuracy of injection, and the amount of injection was decreased to 200nL. In 1 out of the 8 animals receiving the smaller injection, the lesion of the GPi was not complete and lesioned-related performance degradation could not be observed.

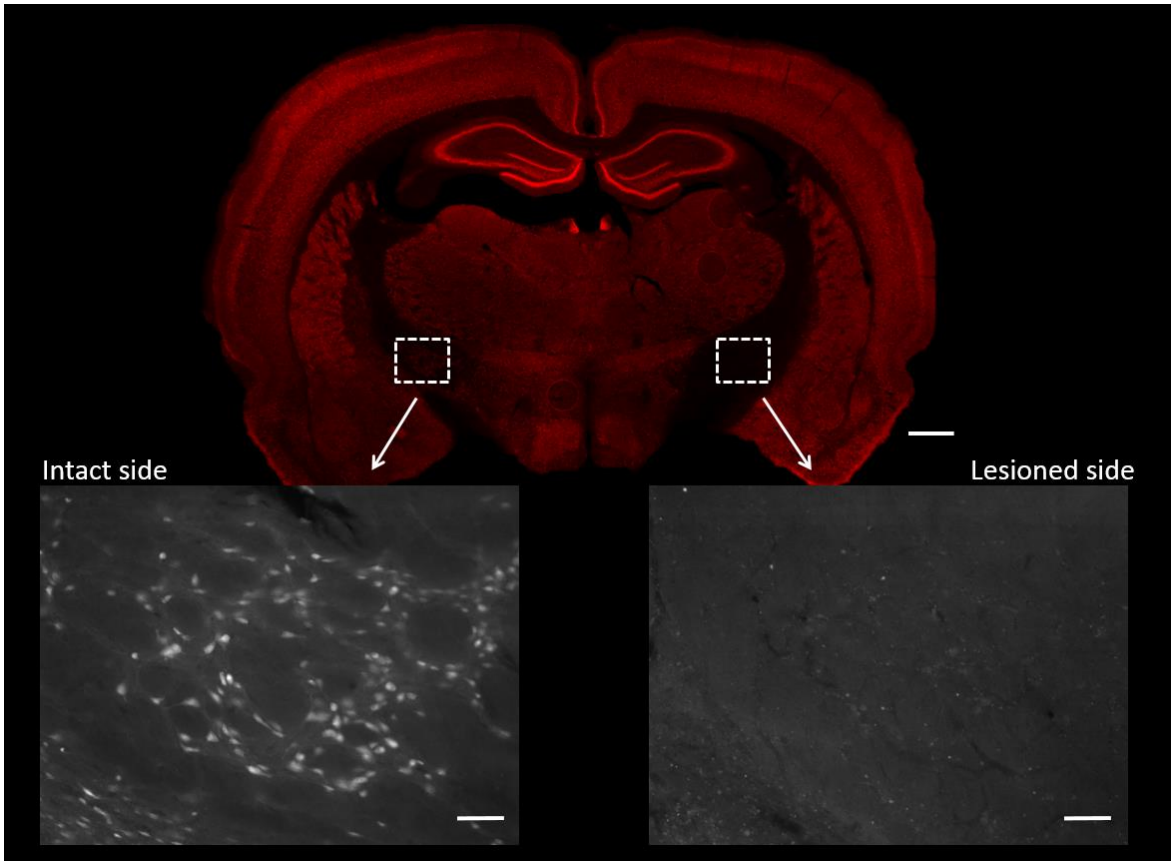

Figure 3.1 Immunohistology verifying GPi lesion. (Top) Immunohistochemistry for the neuron-specific nuclear protein (NeuN) of a rat with left GPi lesioned. White dotted ovals depict the location of GPi in both hemispheres. Scale bar: 1mm. (Bottom left) Magnification (10X) of the intact side showing neurons. (Bottom right) Magnification (10X) of the lesioned side. Scale bars for 10X images: 0.1mm.

Result

All 21 animals trained on the two-press task converged on 700-ms inter-press intervals (IPIs) and passed the learning criteria (Methods in Chapter 2) before unilateral lesion of the GPi.

To probe whether the GPi is required for the motor task, we lesioned it after animals had reached asymptotic performance. Similar to the striatal lesions in Chapter 2, lesions were performed on the hemisphere contralateral to the forepaw used for the first lever-press in a sequence. The region and size of the GPi lesions (Figure 3.1) were comparable to previous studies (Lütjens, Krauss, and Schwabe 2011; Henderson et al. 2006) for both the normal injection of 400nL excitotoxin and the smaller injection of 200nL. Histology was performed to ensure that the GPi was lesioned in all animals included in the analysis. In general, it was easier to have a clean, complete lesion of the GPi compared to striatal lesions because the GPi is a relatively small nucleus within the internal capsule isolated from other brain nuclei. In only one animal, the lesion was incomplete. This animal did not show the performance degradations to be discussed, and it was removed from the analysis. For all other animals, the lesions were complete, yielding histology similar to Figure 3.1.

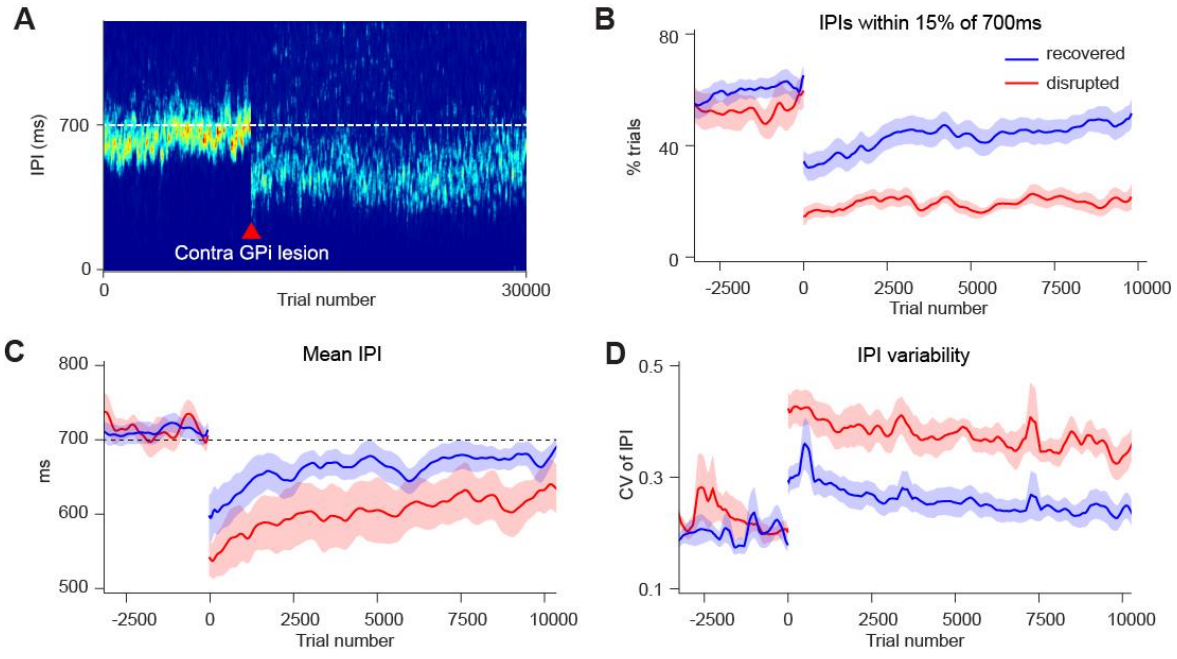

Figure 3.2 Contralateral GPi lesions disrupted performance, but two-thirds of the animals recovered after re-training. (A) Density plot of the IPI distribution before and after contralateral GPi lesions from a rat in the disrupted group. White dotted line denotes the 700-ms target. (B) Percentage of trials with IPI within 15% of 700ms before and after lesions. n=14 for recovered group and n=7 for disrupted group. (C) Mean IPI of the population before and after lesions. (D) Mean CV of IPI of the population before and after lesions. All manipulations were done after the animals had reached asymptotic performance on the task. The shaded regions denote SEM across animals.

Similar to the striatal lesions, spontaneous body rotations were occasionally observed on the day after surgery, but resolved within two or three days. After 10 days of recovery, no non-specific deficits in motor control were observed, and behavioral training resumed.

We found that contralateral GPi lesions (n=21) acutely disrupted motor performance. However, in two-thirds of our animals (n=14), the disruption was temporary. If we define performance recovery as the criterion for learning the task (i.e., the mean of IPI is within 10% of target and the CV of IPI is less than 0.25), their performance recovered after 12112 ± 9597 trials of re-training. Given such a major discrepancy between animals that recovered and those that did not (see explanation in Discussion), we will separate the two groups ("disrupted" and "recovered," respectively) in the following analysis.

After GPi lesions, performance degraded in both the disrupted group (n=7, Figure 3.2A) and recovered group (n=14). The percentage of trials with IPIs within 15% of the 700-ms target dropped significantly from 53.9% ± 13.0% to 18.7% ± 4.4% for the disrupted group ($p = 4.5 \times 10^{-4}$) and from 60.8% ± 10.1% to 37.9% ± 14.7% for the recovered group ($p = 1.0 \times 10^{-5}$. Figure 3.2B). Such degradation was not observed in control animals after taking 10 days of rest without training (Before rest: 63.9% ± 8.6%; after rest: 63.2% ± 13.0%. p = 0.80). After 10,000 trials of training, the same indicator stayed around 21.9% ± 8.9% for the disrupted group (p = 0.0045 relative to pre-lesion performance). On the other hand, the recovered group improved to 48.4% ± 16.5%, but did not fully recover compared to its pre-lesion performance (60.8% ± 10.1%, p = 0.0058).

The performance deficits related to both the mean IPI (Figure 3.2C) and timing variability (Figure 3.2D). For the recovered group, the mean IPI dropped from 715 ± 41ms to 669 ± 58ms immediately after lesion (p = 0.011 relative to pre-lesion, p = 0.43 relative to control) and returned to 688 ± 41ms after 10,000 trials (p = 0.034 relative to pre-lesion). The timing variability, measured as CV of IPI, increased from 0.21 ± 0.07 to 0.28 ± 0.08 after lesion (p = 0.031 relative to pre-lesion, p = 0.18 relative to control) and

decreased to 0.26 ± 0.08 after 10,000 trials (p = 0.079 relative to pre-lesion). For the disrupted group, the mean IPI dropped from 725 ± 36ms to 598 ± 102ms immediately after lesion (p = 0.032 relative to pre-lesion, p = 0.064 relative to control) and stayed around 623 ± 67ms after 10,000 trials (p = 0.0041 relative to pre-lesion). The CV of IPI almost doubled from 0.21 ± 0.02 to 0.39 ± 0.08 after lesion (p = 0.00076 relative to pre-lesion, p = 0.0042 relative to control) and stayed around 0.35 ± 0.07 after 10,000 trials (p = 0.0017 relative to pre-lesion). Overall, the results suggest that GPi lesions disrupted performance, but some animals could recover, possibly due to redundancy of the basal ganglia outputs (see Discussion).

The delay time to the next lever press after unrewarded IPIs was also affected in both the disrupted and recovered groups, but more severely affected in the former (Figure 3.3A). For the disrupted group, the percentage of trials with delay time above 1.2s decreased from 68.1% ± 9.1% to 36.3% ± 4.6% (p = 0.0094 in paired t-test, 500 trials, Figures 3.3B) accompanying a drop of two-press trials from 73.7% ± 5.1% to 35.7% ± 2.8% (p = 0.0003, 500 trials, Figure 3.3D). For the recovered group, the percentage of trials with delay time above 1.2s across animals decreased from 62.0% ± 4.4% to 51.5% ± 6.4% (p = 0.028, Figures 3.3B) accompanying a drop of two-press trials from 72.3% ± 3.2% to 51.6% ± 4.9% (p = 0.0003, Figure 3.3C). In comparison, the control animals were not affected in both the percentage of trials with delay time above 1.2s (p = 0.89) and the percentage of two-press trials (p = 0.60).

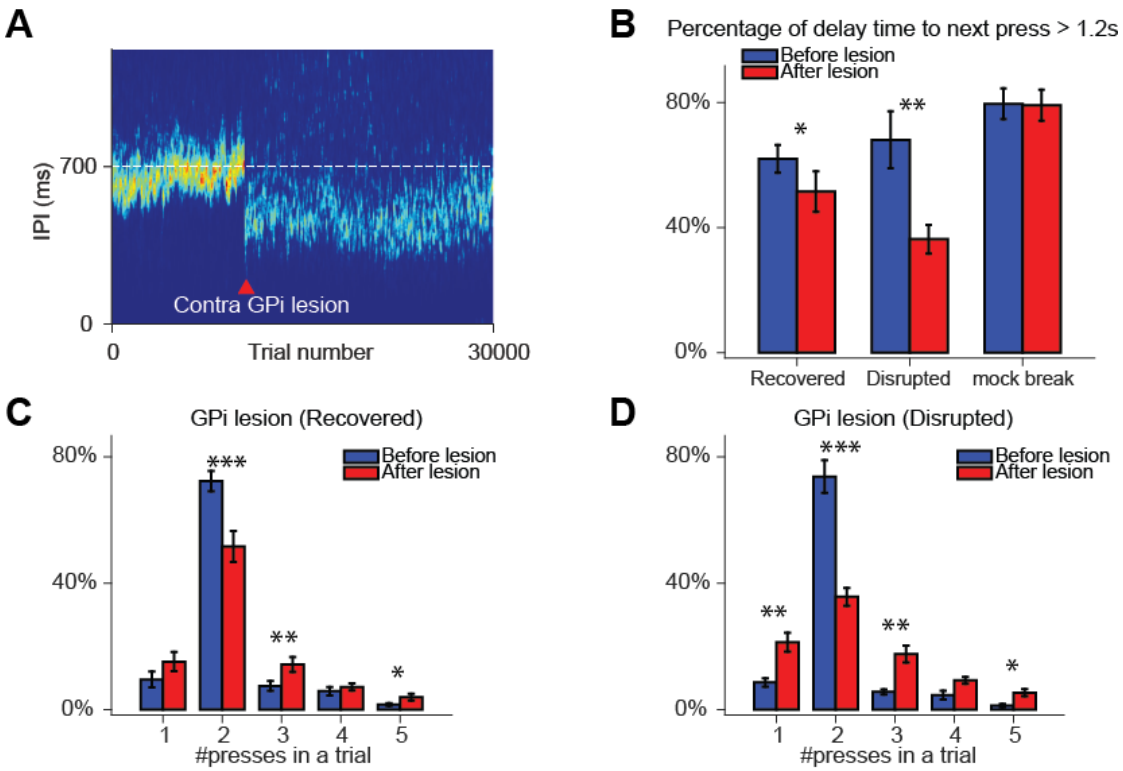

Figure 3.3 Learned structure of inter-trial delay was disrupted by contralateral GPi lesion.
(A) Density plot of the delay to next lever press before and after lesions from a rat in the disrupted group. (B) Percentage of delay time to next press after unrewarded IPIs longer than the predetermined 1.2s (in 500 trials) before and after GPi lesions or mock break. (C and D) Percentage of trials with 1 to 5 lever presses (in 500 trials) before and after lesions in the recovered group (C) and disrupted group (D). All lesions were done after the animals had reached asymptotic performance on the task. Sample numbers are the same as in Figure 3.2. In paired t-tests, '*', '**' and '***' represent $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively. Error bars denote SEM across animals.

As in Chapter 2, we visualized the change in task-related movement patterns by plotting how long the lever was depressed for the first press, relative to how long it took to press the lever a second time (Figures 3.4A and B). In the disrupted group, the centroid shifted by 0.31 ± 0.06 in the parameter space after lesion (relative to 0.09 ± 0.02 in controls, $p = 0.0023$. Figure 3.4C), and the average pairwise distance, measuring cluster dissimilarity, between the pre- and post-lesion distributions was 0.49 ± 0.04 (relative to 0.27 ± 0.03 in controls, $p = 0.0004$, Figure 3.4D). The same indicators were 0.14 ± 0.03 and 0.36 ± 0.02 for the recovered group, respectively, significantly lower than those of the disrupted group ($p = 0.0048$ and

p = 0.0047 for both indicators in t-tests, Figures 3.4C and D) but still higher than those of the control group (p = 0.17 and p = 0.032). The results show that the lesion of the GPi disrupted task-related movement patterns in learned motor sequences.

One interesting question is whether the GPi-lesioned animals recover the same movement patterns after lesion or re-learn the task with new motor sequences. Using the two metrics on lever-pressing time discussed above, the question can be addressed qualitatively. In the disrupted group, the centroid shifted by 0.24 ± 0.12 in the parameter space of lever-pressing durations 10,000 trials after lesion (relative to 0.09 ± 0.02 in controls, p = 0.013), and the average pairwise distance between the pre- and post-lesion distributions was 0.44 ± 0.10 (relative to 0.27 ± 0.03 in controls, p = 0.0029), showing that the movement patterns of these animals were still different from pre-lesion. In the recovered group, however, the first and second metrics were 0.15 ± 0.12 (p = 0.26 relative to controls) and 0.35 ± 0.14 (p = 0.16 relative to controls), closer to those of control animals. Therefore, the animals in the recovered group might have partially recovered their pre-lesion motor patterns.

The ipsilateral GPi may contribute to variability of post-lesion recovery, as the basal ganglia project to downstream motor areas bilaterally (Deniau et al. 1977; Takada et al. 1994; Parent, Lévesque, and Parent 1999). Therefore, we lesioned the ipsilateral GPi in 10 of the 14 recovered animals. Within 10,000 trials after lesions, 8 of the 10 lesioned animals recovered according to the performance criteria. Since 66.7% of the animals (14 out of 21) recovered after contralateral lesions and 80.0% (8 out of 10) recovered after ipsilateral lesions, roughly half (53.4%) of the animals recovered after bilateral GPi lesions. We speculate that other basal ganglia outputs, such as SNr, compensated for the loss of GPi neurons in the recovered animals.

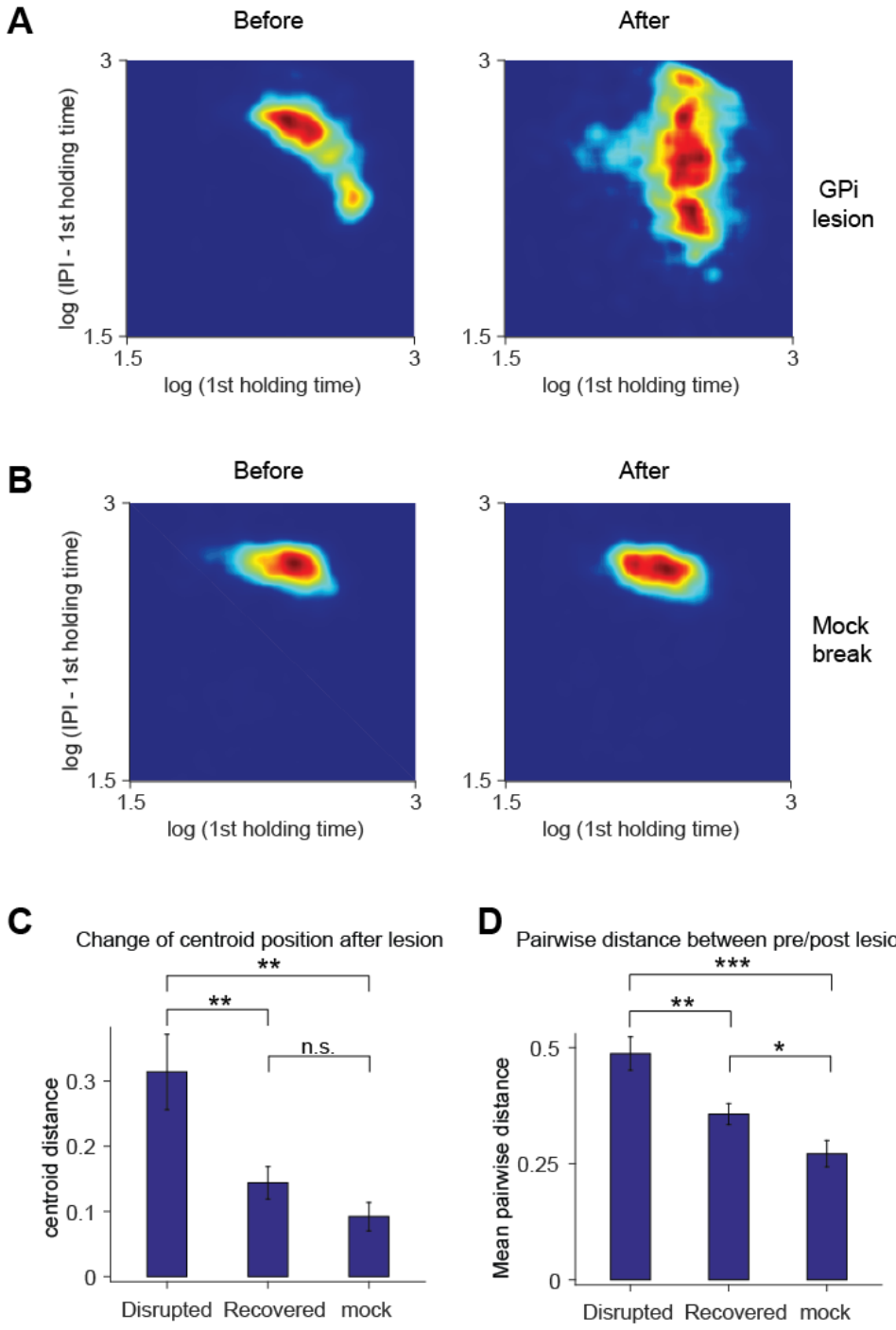

Figure 3.4 Learned movement patterns were disrupted by contralateral GPi lesions.
(A and B) Density plot of the logarithm of the two parameters, 1) the holding time of the first lever press (1st holding time) and 2) the time between the first lever release and the second lever press (IPI – 1st holding time) before and after lesions from a rat in the disrupted group (A) and a rat receiving mock break (B). Each plot consists of 2000 trials. (C) Bar chart showing the population mean of the distances between the two centroids of the clusters plotted above before and after manipulations in 500 trials. (D) Bar chart showing the population mean of the pairwise distances between the two clusters plotted above before and after manipulations in 500 trials. Sample numbers are the same as those in Figures 3.2 and 3.3. In paired t-tests, '*', '**' and '***' represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (n.s represents $p > 0.05$). Error bars denote SEM across animals.

Discussion

To examine the role of the basal ganglia in the execution of learned motor sequences, we lesioned the GPi bilaterally after training and quantified performance degradation. We found that GPi lesions acutely disrupted motor performance. However, in roughly half of the animals the performance partially recovered, possibly due to redundancy in the basal ganglia outputs, as discussed below.

Comparison with previous studies of GPi involvement in motor skill execution

There are relatively few motor-related studies of the GPi. To my knowledge, there have been no studies on the role of the GPi in motor skill execution in rodents except those performed by the Schwabe lab (Lütjens, Krauss, and Schwabe 2011). They lesioned the GPi in rats bilaterally and showed that limb co-ordination was not affected in a rotating rod task, in which animals had to stay on a rotating rod by coordinating the limbs. However, the task does not require training, so the motor skill is different from the learned motor sequences we study.

There was one inactivation study in primates done by Desmurget and Turner (2010). They trained two monkeys to perform sequential reaching movements directed to visual targets. Contrary to our results, muscimol inactivation of the GPi did not affect execution of overlearned sequences, except that the movements became slower. However, the dose of muscimol applied in their inactivation experiments was small (0.5–2.0 µl) and might not have silenced the whole GPi. The discrepancy may further be due to differences in the nature of the task, especially the involvement of instructive cues in training. Different neural substrates are probably required for motor sequence learning with or without cues, as different brain regions are preferentially activated when humans or animals perform movements with or without instructive cues (Mushiake et al. 1991; Elsinger et al. 2006). This is consistent with clinical observations showing that visual cues can improve motor sequence execution in Parkinson's disease patients (Lewis et al. 2000; Azulay et al. 2006; Mak and Hui-Chan 2007).

Implications on performance degradation after DLS lesions

The performance degradation of DLS-lesioned animals described in Chapter 2 could be caused by the direct loss of function or indirect influences of aberrant activity. In this chapter, we address the issue by lesioning the GPi, a common clinical methodology to block basal ganglia pathological activity (Obeso et al. 2009; Lozano et al. 1995; Baron et al. 1996; Mink 1996). The lesions block one of the output streams of the basal ganglia and further examine the role of the basal ganglia in motor sequence execution. Similar to DLS lesions, we find that GPi lesions acutely disrupt performance of learned motor sequences, suggesting that performance degradation caused by DLS lesions (Chapter 2) reflects the function of the basal ganglia rather than simply being the consequence of aberrant striatal activity.

In a preliminary experiment we lesioned the DLS in four animals that had recovered from bilateral GPi lesions. If the hypothesis that DLS lesions produce aberrant basal ganglia activity is true, blocking the basal ganglia output will, at least partially, prevent performance degradation. However, we found that prior GPi lesions did not prevent performance degradation related to DLS lesions. Although a higher sample number is required for confirmation, the result supports the direct involvement of the DLS in motor execution.

Redundancy in the basal ganglia outputs

About half of our animals partially recovered their performances after bilateral GPi lesions. This is consistent with clinical data on human GPi lesions showing variability in motor deficits across patients (Bhatia and Marsden 1994). Such variability can be explained by redundancy of the basal ganglia outputs. The SNr has been considered to be functionally similar to the GPi (Kumar 2014). It receives inputs from structures similar to those providing input to the GPi, including the striatum, subthalamic nucleus, and globus pallidus external segment (Gerfen and Bolam 2010). Despite differences in outputs, both the SNr

and GPi project to the pedunculopontine nucleus, which has been implicated in voluntary limb movements (Tsang et al. 2010). The above similarities of the SNr and GPi suggest that the SNr can potentially replace the GPi when the latter is lesioned.

Remaining questions and future experiments

Lesions of the DLS and GPi described in Chapters 2 and 3 have implicated the basal ganglia in the execution of the cortex-independent motor skills we train. There are two possibilities for the underlying circuitry: 1) The direct basal ganglia-brainstem projections are sufficient for executing complex motor sequences, or 2) the basal ganglia-brainstem-thalamic loops (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995) are necessary for the execution. Lesion or inactivation of the motor thalamus in our task will provide insights into this question, and such experiments are now underway in the lab.

Chapter 4 Role of striatum in motor sequence learning

Introduction

In chapters 2 and 3 I showed that the basal ganglia are required for executing learned motor sequences. In this chapter I will examine whether the basal ganglia also are involved in the initial acquisition of motor sequences by lesioning the striatum before training on the task.

As described in the introductory chapter, the striatum has been implicated in motor-sequence learning. For example, Parkinson's disease patients are more likely to have deficits in learning sequences (Pascual-Leone et al. 1993; Jackson et al. 1995; Helmuth, Mayr, and Daum 2000), possibly due to aberrant activity in the striatum induced by partial loss of basal ganglia neurons (Mink 1996; Vitek and Giroux 2000; Okun and Vitek 2004). Functional magnetic resonance imaging and positron emission tomography have revealed the activation of the striatum during motor-sequence learning (Julien Doyon et al. 2006; Grafton, Hazeltine, and Ivry 1995; Rauch et al. 2004; Rauch et al. 1998). In animal studies, striatal activity is dynamically modified during motor-skill learning and habit formation (Yin et al. 2009; Barnes et al. 2005; Costa et al. 2004).

However, many of these studies involved only electrophysiological or functional imaging experiments, demonstrating a correlation between neural activity and functionality (Henson 2005; Paus 2005; Brown and Eyler 2006; Weber and Thompson-Schill 2010; Humphrey 2000; Sporns 2010). Therefore, lesion studies are necessary for establishing causality between the dynamics in a brain region and behavior (Mcgeer, Olney, and Mcgeer 1978). Moreover, compared with studies on basal ganglia diseases, controlled lesions in experimental animals can provide cleaner and more interpretable results.

In Chapter 2, we hypothesized that the motor cortex tutors the basal ganglia via its projections to the DLS during learning. This hypothesis is supported by functional and anatomical evidence. (1) Our lab

has shown that the motor cortex is required for learning but not executing non-dexterous motor sequences (Kawai et al. 2015). (2) According to multiple tracing studies, the motor cortex projects heavily to the DLS (Figure 2.7D in Chapter 2; see also Shepherd 2013; Doig, Moss, and Bolam 2010), making it a plausible target of tutoring by the motor cortex and, hence, it may be essential for learning our task.

Alternatively, the DMS could be involved in learning motor sequences even though it is largely dispensable for executing the motor skill we train. Imaging and inactivation studies have suggested that the early, goal-directed phase of learning involves the DMS and prefrontal cortex (Miyachi et al. 1997; Jog et al. 1999; Rémy et al. 2008; Jueptner et al. 1997; Puttemans, Wenderoth, and Swinnen 2005; Ma et al. 2010; Floyer-Lea and Matthews 2005; J Doyon et al. 1996; Grafton, Hazeltine, and Ivry 1995; Seitz et al. 1990; Jenkins et al. 1994; Toni et al. 1998; Robertson 2007). When the skill is learned and becomes automatic, it is believed to become more dependent on the DLS and motor cortex. In Chapter 2, we showed that the DLS is indeed important for executing the skill we train after it has been acquired. Now, we seek to know the extent to which the DMS and the DLS are critical for the early phase of learning.

To test whether the DLS, DMS or both are essential for learning motor sequences, we examined how pharmacological lesions of these structures affected learning in the two-press training paradigm described in previous chapters. In contrast to the canonical view that DMS is important for early phase of learning, we find that the DLS but not the DMS is essential for learning our task.

Method

Behavioral training and lesion surgeries

The behavioral task is the same as described in Chapter 2. Before training, the DLS (n = 4) or DMS (n = 4) were lesioned bilaterally. After surgery, animals were allowed to recover in the animal facility for 10 days before starting behavioral training.

See Methods in Chapter 2 for details on the training procedure and protocol for lesioning the DLS and DMS. However, the animals in this experiment were given two 1-hour training sessions per day (instead of three 1-hour training sessions as described in Chapter 2).

Learning criterion (adopted from Kawai et al. 2015):

Criterion performance: The major goals of learning the task are (1) reaching the IPI target of 700 ms and (2) lower the variation of the IPIs. To quantitatively define the goals, animals were deemed to have reached criterion performance on the task when, for a 3,000-trial sliding window, the CV was less than 0.25 and the mean of the IPI distribution was within 10% of the target.

Learning the prescribed 1.2-s inter-trial delay: According to our definition, the prescribed 1.2-s inter-trial delay was learned when, for a 3,000-trial sliding window, the median time to the next tap was larger than 1.2 s and this was maintained for at least another 3,000 trials. Intervals >5 s, or those that occurred after rewarded trials, were excluded (but included for the sliding window).

Result

To probe the role of the striatum in learning motor sequences, we lesioned the DLS and DMS bilaterally in untrained rats (n = 4 for both groups). The average lesion volumes of the DMS group and the DLS group were comparable (5.70 ± 0.92 mm^3 for DMS and 5.30 ± 0.44 mm^3 for DLS, p = 0.70 in t-test. See Method in Chapter 2). The regions lesioned (Figure 4.1) were similar to those of previous studies (Castañé, Theobald, and Robbins 2010; Featherstone and McDonald 2004; Yin, Knowlton, and Balleine 2004). After 10 days of recovery, no major deficits in motor control were observed, and behavioral training began in home cages.

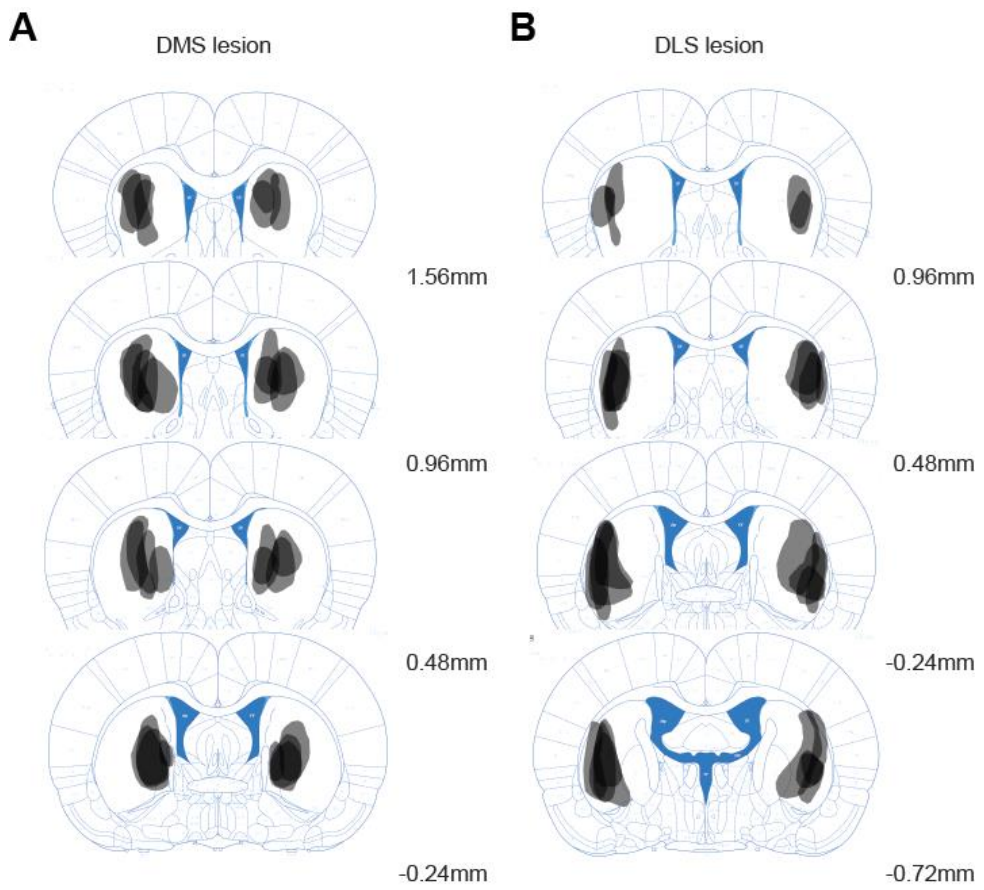

Figure 4.1 Histology of DLS and DMS lesions. Schematics of a series of coronal sections illustrating the extent of DMS (A) and DLS (B) lesions. The level of greyness represents coincidence of lesions from different animals. The diagrams are based on a rat brain atlas (Paxinos and Watson, 2007). The numbers indicate distance in mm from bregma. See Figure 2.7 of Chapter 2 for photomicrographs of nissl-stained slices from an example striatal lesioned rat.

Early performances were comparable across the DLS and DMS animals

Early in training, DLS-lesioned, DMS-lesioned and control (intact) groups showed comparable task performance (Figure 4.2). In the first 1,000 trials of two-press training, one-way ANOVA tests revealed that the mean and CV of the inter-press interval distribution (IPIs) were not significantly different for the three groups (Mean of IPI: $F_{2,23} = 0.29$, $p = 0.75$. CV of IPI: $F_{2,23} = 1.14$, $p = 0.34$. Figures 4.2A and B). The fraction of IPIs within 10% of the 700-ms target was also similar ($F_{2,23} = 2.28$, $p = 0.12$. Figure 4.2C). Furthermore, the number of lever-presses in a training session, reflecting motivation to do the task, were also not significantly affected by either DLS or DMS lesions ($F_{2,23} = 0.2$, $p = 0.82$. Figure 4.2D). The similarity

of the above performance metrics during early learning suggest that the lesioned animals are motivated and capable of pressing the lever, suggesting that there is no obvious motivational or motor control deficit that would prevent them from learning the task.

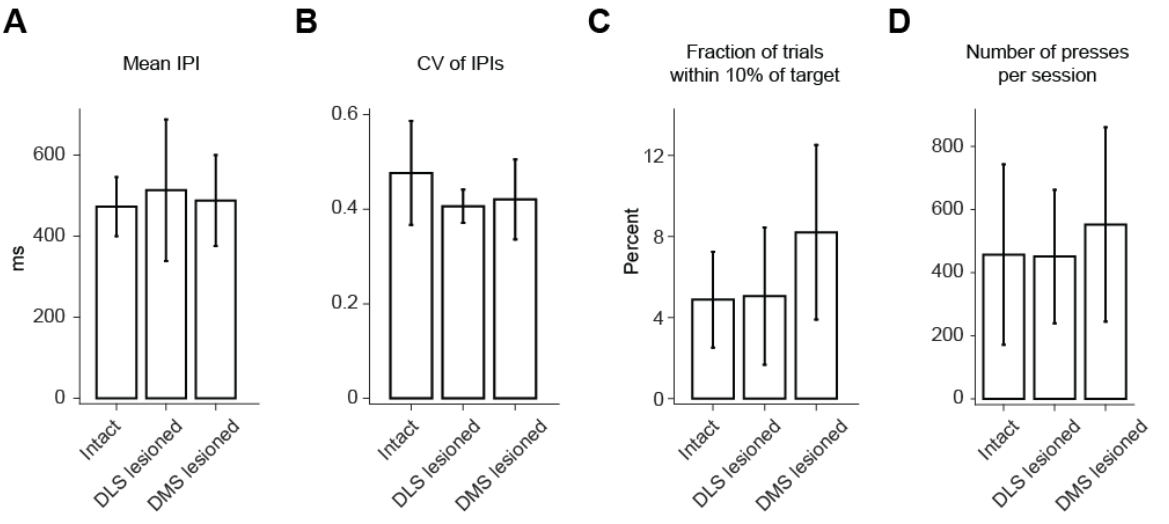

Figure 4.2 Performance metrics were comparable across the DLS, DMS and intact groups during early learning. Lesioned rats did not show any apparent motivational or task-relevant motor control deficits early in learning (first 1,000 trials for A, B and C). (A) Mean of the IPIs. (B) CV of the IPIs. (C) Fraction of trials within 10% of target. (D) Number of lever presses per session (first 6 sessions). Error bars denote SD across animals. In one-way ANOVA, $p > 0.05$ between groups for all four figures.

DLS but not DMS is required for learning the cortex-independent motor skill

Despite non-obvious deficits (Figure 4.2), DLS lesions severely disrupted learning in our task (Figures 4.3A and B). None of the DLS animals (n = 4) learned the task to criterion performance. After 30,000 trials of training, the mean IPI across the population (486ms ± 78ms) was significantly lower than the DMS group (708ms ± 4ms, p = 0.0012, Figure 4.3C) and intact group (684ms ± 64ms, $p = 5.5 \times 10^{-5}$). The timing variability, measured by the CV of the IPIs (0.47 ± 0.11), was over two times that of DMS (0.21 ± 0.05, p =

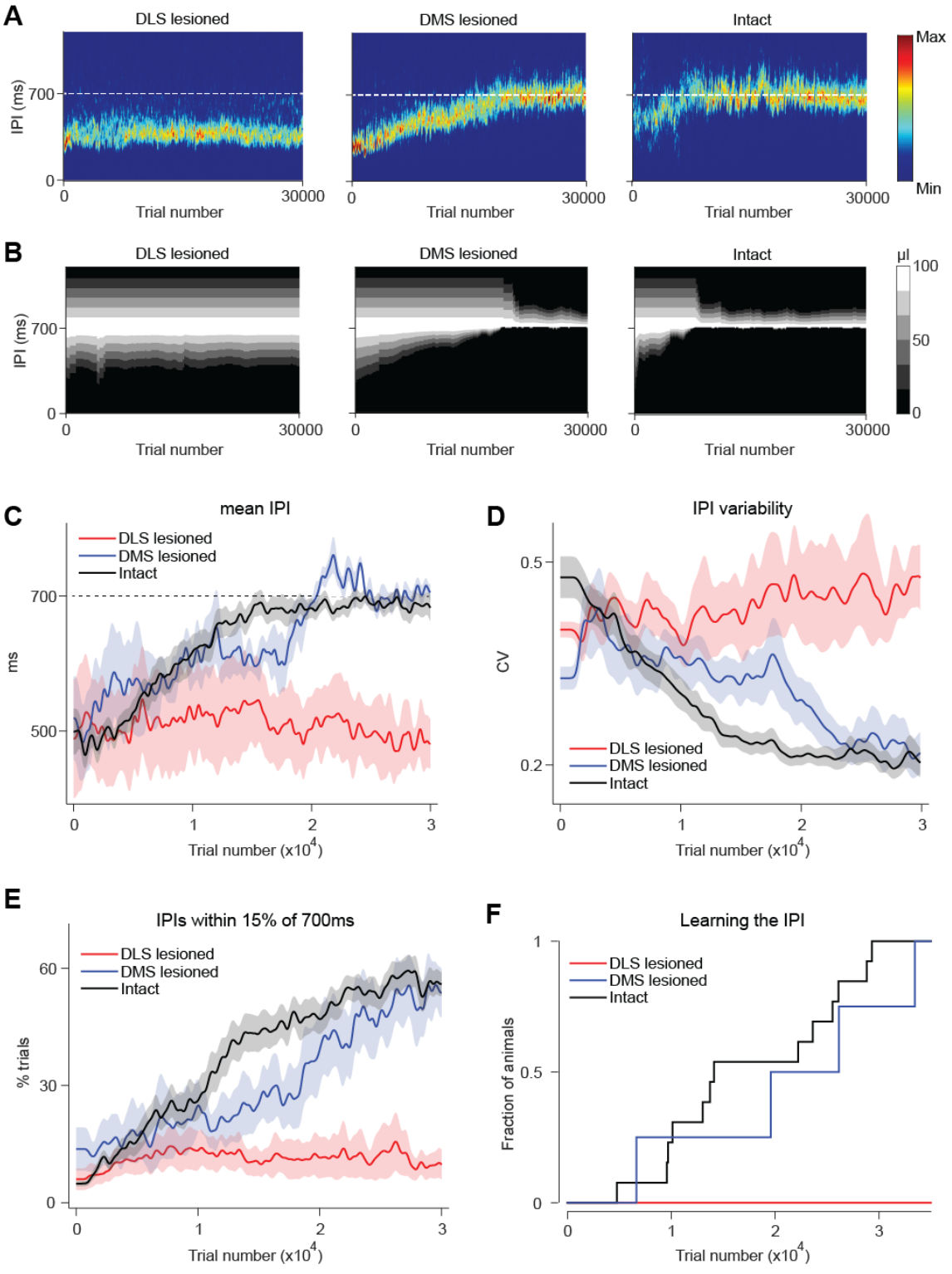

Figure 4.3 The DLS but not the DMS is required for learning the cortex-independent motor skill we train (see next page for details).

Figure 4.3 (continued). The DLS but not the DMS is required for learning the motor cortex-independent motor skill.
(A) Density plots of the IPI distribution for rats with the DLS lesioned (left), the DMS lesioned (middle), or no lesions (right). White dotted lines denote the 700-ms target. (B) Graded reward landscape in (A). Amount of water rewarded was automatically adjusted based on IPI to ensure a reward rate of ~35%. (C, D and E) Learning curves showing the mean IPI (C), CV of IPI (D) and percentage of trials with IPIs within 15% of target (E) as a function of training across all rats. Shaded regions denote SEM. (F) Cumulative histograms showing the fraction of animals that learned the task to criterion performance (see method) as a function of training.

0.0054, Figure 4.3D) and intact animals (0.21 ± 0.09, $p = 1.1 \times 10^{-4}$). Neither criteria improved compared to the first 1,000 trials of training (mean IPI: 515ms ± 172ms, p = 0.70; CV of IPI: 0.40 ± 0.04, p = 0.40). The inability to learn was also directly reflected in the percentage of IPIs within 15% of the 700-ms target (9.3% ± 5.5%, Figure 4.3E), which was significantly lower than that of DMS (53.9% ± 5.0%, p = 0.0028) and intact animals (54.8% ± 9.2%, $p = 6.9 \times 10^{-14}$). Since it was possible that it simply took longer for the lesioned animals to learn, we kept 2 of the 4 animals in training three times longer (over 60,000 trials) than it took intact animals to learn the skill. They still did not master the task according to our criterion.

In contrast, DMS lesions did not abolish learning, even though the learning rate was slightly slower as compared to the intact group (Figures 4.3A and B). After 30,000 trials of training, the mean IPI (DMS: 708ms ± 4ms, control: 684ms ± 64ms, p = 0.48), CV of IPIs (DMS: 0.21 ± 0.05, control: 0.21 ± 0.09, p = 0.90) and percentage of trials with IPIs within 15% of target (DMS: 53.9% ± 5.0%, control: 54.8% ± 9.2%, p = 0.86) were all comparable between the DMS animals and controls (Figures 4.3C, D and E). On average, DMS rats took 21,352 ± 11,581 trials to achieve criterion performance, around 20% longer than the controls (17,735 ± 8,431 trials, Figure 4.3F) even though the difference was not statistically significant (p = 0.50). A longer learning time in the DMS animals could be potentially caused by partial DLS lesions in the DMS-lesioned animals, given that the exact anatomical boundary between the DMS and DLS is unclear.

Furthermore, the DLS animals did not learn to withhold lever pressing for the predetermined 1.2s after unrewarded IPIs (Figures 4.4A, see Method for learning criteria). The fraction of unrewarded IPIs

that were followed by a delay of more than 1.2s was 33.3% ± 15.9% after 30,000 trials (Figure 4.4B), significantly lower than that of intact animals (61.4% ± 19.0%, p = 0.013). The percentage of two-press trials after training, which correlates with inter-trial delay, was also lower in DLS animals (DLS: 45.1% ± 7.1%, intact: 69.3% ± 9.3%, p = 9.9 X 10^{-5}, Figure 4.4C). Neither criteria improved in the DLS animals as compared with the first 1,000 trials of training (Fraction of unrewarded IPIs over 1.2s: 22.2% ± 8.1%, p = 0.17; Percentage of two-press trials: 38.9% ± 7.8%, p = 0.29).

On the other hand, all DMS animals were able to learn the 1.2s-inter-trial. Both the fraction of unrewarded IPIs over 1.2s (DMS: 65.0% ± 14.8%, intact: 61.4 ± 19.0%, p = 0.73, Figures 4.4A and B) and the percentage of two-press trials (DMS: 71.0% ± 4.4%, intact: 69.3% ± 9.3%, p = 0.73, Figure 4.4C) were comparable with the control animals. However, the DMS animals took longer, though not significantly, than the controls to learn it (DMS: 20,668 ± 5,513 trials, intact: 16,233 ± 12,583 trials, p=0.51, Figure 4.4D).

Discussion

To examine the role of the striatum in learning new motor sequences, we pharmacologically lesioned the DLS and DMS before training rats to perform spatiotemporally precise lever-press sequences. Remarkably, DLS lesions severely disrupted learning in terms of motor timing (Figure 4.3) and trial structure (Figure 4.4). DMS lesions, on the other hand, slightly slowed down but did not abort learning. The results suggest that the DLS is a potential subcortical target for the motor cortex's tutoring function.

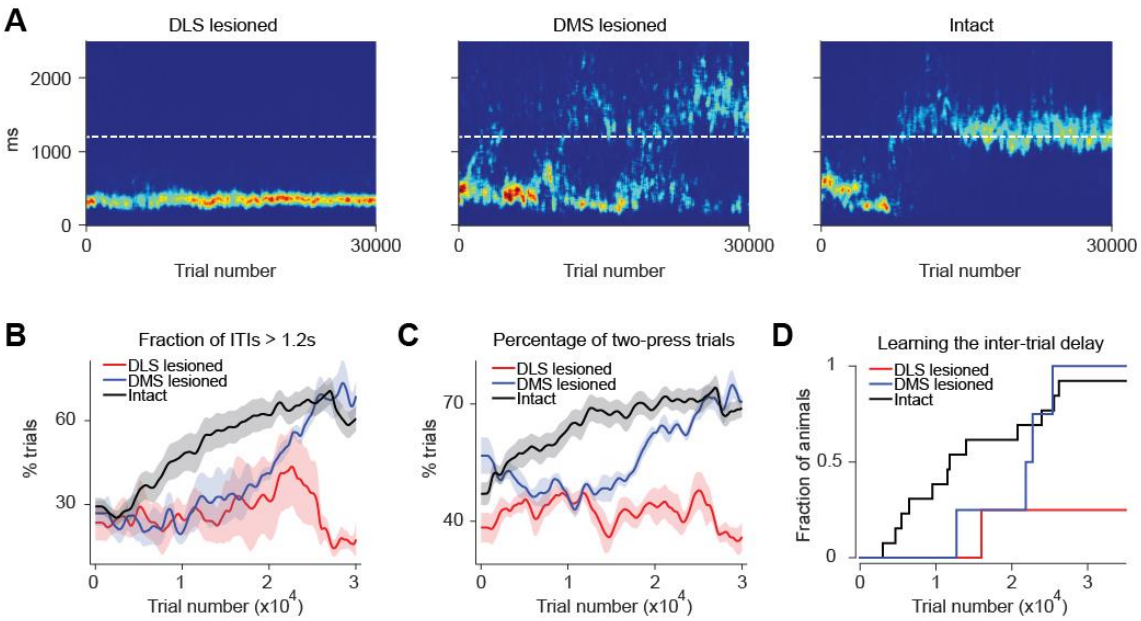

Figure 4.4 DLS lesions disrupted learning of inter-trial delay, while DMS lesions only slowed down learning. (A) Density plots of the delay to next lever press in rats with DLS lesions (left), DMS lesions (middle) and no lesions (right). (B and C) Learning curves showing the fraction of inter-trial intervals over 1.2s (B) and percentage of two-press trials (C) as a function of training across all rats. Shaded regions denote SEM. (D) Cumulative histograms showing the fraction of animals that learned the inter-trial delay (see text for criteria) as a function of training.

Comparisons with prior striatal studies on motor sequence learning

This chapter shows that learning our motor task is disrupted by DLS, but not DMS, lesions. The results may seem inconsistent with previous studies showing that the DMS is involved in early learning and the DLS in late learning. This can be explained by the inherent differences in the tasks. For instance, the Hikosaka lab revealed that the DMS analog in monkeys is required for early learning of a visuomotor task (Miyachi et al. 1997). The involvement of externally guided cues possibly recruit the prefrontal and posterior parietal cortices and their target, the DMS (Hikosaka et al. 2002). Likewise, many imaging studies in humans may depend more on the prefrontal cortex and DMS during early learning as external cues and verbal instructions are provided (Rémy et al. 2008; Jueptner et al. 1997; Puttemans, Wenderoth, and Swinnen 2005; Ma et al. 2010; Floyer-Lea and Matthews 2005; J Doyon et al. 1996; Grafton, Hazeltine, and Ivry

1995; Seitz et al. 1990; Jenkins et al. 1994; Toni et al. 1998; Robertson 2007). In contrast, the self-initiated motor sequences in our study are learned via trial-and-error without external cues. This could explain why the DLS but not DMS lesions disrupted learning in our task.

The above speculation is supported by another recording study performed in rats learning a T-maze task instructed by auditory and tactile cues (Thorn et al. 2010). During learning, the DLS developed ensemble spike activity that was heightened at the action boundaries, whereas the DMS developed heightened activity when the animals chose between alternate actions based on cues instructing the correct T-maze arm. This suggests that a task without instructional cues may not require the DMS.

DLS as the tutoring target of the motor cortex

Our results suggest that the DLS may be a direct target for tutoring by the motor cortex. First, the motor cortex is not required for generating the motor skill we train, implicating the subcortical substrates in execution (Kawai et al. 2015). Next, we showed that the basal ganglia, particularly the sensorimotor part, is involved (Chapters 2 and 3). Third, both the motor cortex (Kawai et al. 2015) and the DLS (Chapter 4) are essential for learning, raising the possibility that the DLS is tutored by the motor cortex during learning. This hypothesis is further supported by anatomical studies that reveal strong projections from the motor cortex to the DLS (Shepherd 2013; Doig, Moss, and Bolam 2010).

Potential roles of the DMS in skill learning

Although DMS lesions barely affected learning in our task, it does not preclude the DMS being important for learning other motor skills. The DMS receives projections from the prefrontal, auditory and sensory cortices. Thus, motor tasks that rely more on working memory or sensory feedback may indeed require the DMS for learning. Two classic examples are the visuomotor task in the Hikosaka lab (Miyachi et al. 1997) and T-maze procedural task in the Graybiel lab (Thorn et al. 2010), as discussed previously.

Remaining questions and future experiments

Though we showed that the motor cortex and DLS are both required for learning our motor task, there are important questions left to address. To prove that the DLS is the direct tutor target of the motor cortex, our lab has been inactivating motor cortex-DLS projections in behaving animals to prove causality. We also record from identified striatal projecting motor cortex neurons in learning rats to identify the neural correlates of tutoring.

Chapter 5 Role of primary and secondary motor cortices in motor sequence learning

Introduction

Our lab has shown that rats without motor cortex can execute learned motor sequences (Kawai et al. 2015). However, lesioning the motor cortex before training disrupts learning (Figure 1.2). In our previous studies, we lesioned both the primary and secondary regions (M1 and M2) of the forelimb motor cortex.

In rodents, the part of the motor cortex related to forelimb movements is composed of the caudal forelimb area (M1) and rostral forelimb area (M2). Similar to its primate analog, the rodent M1 receives strong projections from the ventrolateral nucleus of the thalamus (motor thalamus) (Donoghue and Parham 1983), and projects directly to the spinal cord and other subcortical targets (Donoghue and Wise 1982; Miller 1987). Movements can be evoked by M1 microstimulation (Donoghue and Wise 1982; Sanderson, Welker, and Shambes 1984; Neafsey et al. 1986). On the other hand, it has been suggested that the M2 is homologous to the premotor cortex and supplementary motor area (Donoghue and Wise 1982; Sanderson, Welker, and Shambes 1984; Neafsey et al. 1986; Reep, Goodwin, and Corwin 1990). It sends projections to the M1 (Donoghue and Parham 1983; Reep et al. 1987, 1987), the spinal cord (Miller 1987) and other subcortical structures. However, higher current stimulation is required to evoke movement from the M2 compared to the M1 (Donoghue and Wise 1982; Sanderson, Welker, and Shambes 1984; Neafsey et al. 1986).

Despite anatomical differences, studies rarely differentiate the M1 and M2 in experiments (see Chapter 1 for introduction). In a rodent lever-pressing task, the Yin lab has shown that the M2, but not the M1, is required for learning the serial order of two lever presses on two separate levers (Yin 2009). In primates, lesion of the M2 (premotor cortex) also impairs acquisition of new sequences of arm movements (Thaler et al. 1995; Chen et al. 1995). Still, the role of the M1 and M2 in learning motor sequences which do not require the motor cortex for execution (Kawai et al. 2015) has not been studied.

Chapters 2 and 4 implicated motor cortex-DLS projections in the learning and execution of the motor skills we train. Therefore, the motor cortical areas with strong projections to the DLS are possibly involved in learning. As both the M1 and M2 project heavily to the DLS (Van Waes et al. 2012; Voorn et al. 2004; Cospito and Kultas-Ilinsky 1981; McGeorge and Faull 1989), the question is whether they are redundant or if both are needed for learning.

To test the hypothesis, we pharmacologically lesioned the M1 or M2 in animals before training the same motor task discussed in previous chapters. Though neither the M1 nor M2 lesions abort learning, learning rates, comparable between the two groups, are slower than the intact group.

Method

The behavioral task, training procedure and criterion performance are the same as those described in Chapter 4 (Method), where we examined the role of the DLS and DMS in learning.

Before training, the M1 (n = 6), M2 (n = 6) or motor cortex (M1 + M2, n = 11) were lesioned bilaterally by injecting 1% ibotenic acid (82.8nL per site). The coordinates in millimeters (Paxinos and Watson 2007), relative to bregma (anteroposterior, mediolateral, dorsoventral), were as follows: M1: (+1.0, 2.0, -1.5), (+1.0, 2.0, -0.75), (+1.0, 4.0, -1.5) and (+1.0, 4.0, -0.75); M2: (+3.5, 2.25, -1.5), (+3.5, 2.25, -0.75); motor cortex: (+1.0, 2.0, -1.5), (+1.0, 2.0, -0.75), (+3.0, 2.0, -1.5), (+3.0, 2.0, -0.75), (+1.0, 4.0, -1.5) and (+1.0, 4.0, -0.75). After injection, the pipette stayed in the injection site for 5 minutes to allow drug diffusion. The pipette was then pulled out from the brain tissue slowly to prevent drug backflow. After surgery, animals were allowed to recover in the animal facility for 10 days before resuming behavioral training in their home cages.

One-way ANOVA and Tukey HSD tests were used to determine whether there were any significant differences between the means of 3 or more experimental groups.

Result

To investigate the role of the M1 and M2 in motor-sequence learning, we lesioned each area bilaterally in untrained rats (n = 6 for both groups). Figure 5.1 shows a schematic and examples of the lesions. Lesion sizes were comparable to those in previous studies (Yin 2009; Sul et al. 2011). Tissue in the dorsal striatum beneath the M1 and M2 was spared. No major non-specific deficits in motor control were observed 5 days after surgery. Behavioral training in home cages was initiated after 10 days of recovery.

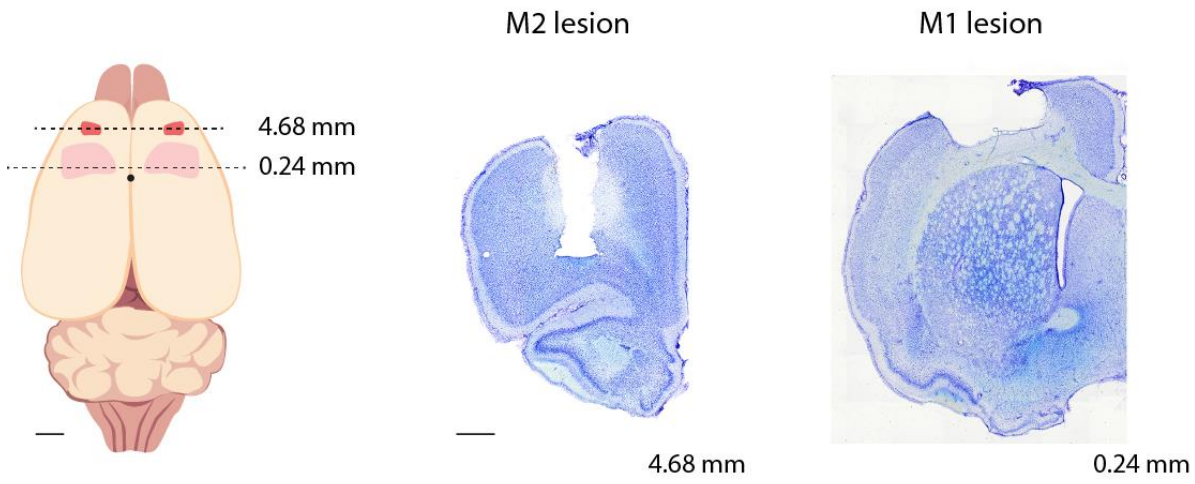

Figure 5.1 Bilateral M1 and M2 lesions.
Left: schematic showing the outlines of the primary (pink) and secondary (red) forelimb motor cortex on both hemispheres. Scale bar: 2mm. Middle and right: Cresyl Violet-stained coronal sections showing the induced lesions in one hemisphere. Different from striatal lesions (Figure 2.7), cortical lesions with excitotoxin often cause more obvious loss of brain tissue in Cresyl Violet-stained sections. The sections are 4.68mm and 0.24mm anterior of bregma respectively for M2 and M1 lesions. Scale bar: 1mm.

Early performance was comparable across the M1, M2, MC and intact groups

Early in training, the M1, M2, motor cortex (M1+M2) and the control (intact) groups had comparable task performance. The mean and CV of the inter-press intervals (IPIs) were not significantly different across all groups in the first 1,000 trials of two-press training (One-way ANOVA: $F_{3,37} = 2.18$, $p = 0.11$ for mean of IPIs; $F_{3,37} = 0.42$, $p = 0.74$ for CV of IPIs. Figures 5.2A and B). Similarly, the fraction of IPIs within 10% of the 700-ms target was comparable across groups ($F_{3,37} = 2.63$, $p = 0.064$. Figure 5.2C). Moreover, motivation for the task, as measured by the number of lever-presses in a training session, was not affected by the lesions ($F_{3,37} = 0.49$, $p = 0.69$. Figure 5.2D). The similarity of early performance ensured fair comparisons of learning rates between lesioned animals and controls.

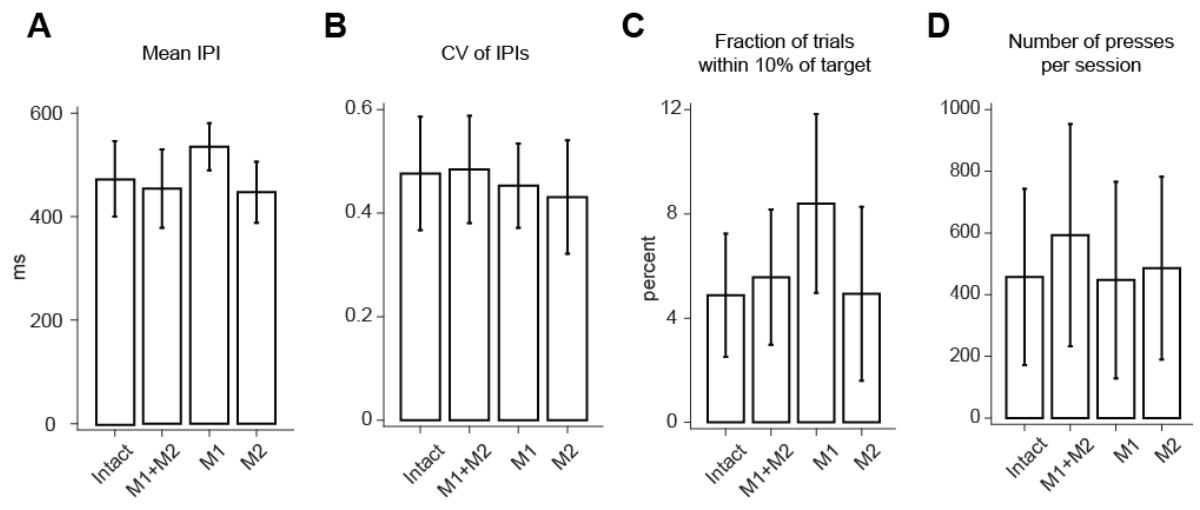

Figure 5.2 Performance metrics were comparable across the M1, M2, MC and intact groups during early learning. Lesioned rats did not show any apparent motivational or task-relevant motor control deficits early in learning (first 1,000 trials for A, B and C). (A) Mean of the IPIs. (B) CV of the IPIs. (C) Fraction of trials within 10% of target. (D) Number of lever-presses per session (first 6 sessions). Error bars denote SD across animals. In one-way ANOVA, $p > 0.05$ between groups for all four figures.

Lesions of the M1 or M2 slowed down but did not abort learning

We expected that lesioning the M1 or M2 would disrupt learning because rats cannot learn the task after the M1+M2 lesions (Figure 5.3, and Kawai et al. 2015). Surprisingly, neither the M1 nor M2 lesions abolished learning (Figures 5.3A and B). After 30,000 trials of training, the mean IPI across the population of both the M1 and M2 groups (M1: 703ms ± 36ms, M2: 693ms ± 56ms) was close to the 700-ms target, comparable to controls (684ms ± 64ms, p = 0.89 between M1 and intact animals, p = 0.99 between M2 and intact animals. Figure 5.3C). Likewise, the timing variability (CV of IPIs) of M1 and M2 animals (M1: 0.21 ± 0.09, M2: 0.21 ± 0.07) was similar to controls (0.21 ± 0.09, p = 1.00 between the M1 and intact animals, p = 1.00 between the M2 and intact animals. Figure 5.3D). The ability of lesioned animals to learn the target IPI and reduce the CV of the IPI distribution was also reflected in the percentage of IPIs within 15% of 700-ms target (M1: 50.6% ± 21.2%, M2: 55.3% ± 15.8%, control: 54.8% ± 9.2%. p = 0.88 between the M1 and intact animals, p = 1.00 between the M2 and intact animals. Figure 5.3E). In contrast, the above performance metrics of M1+M2 lesioned animals (mean IPI: 577ms ± 47ms, CV of IPI: 0.38 ± 0.04, percentage of IPIs within 15% of target: 23.0% ± 5.2%, n=11 rats) were significantly poorer than the other groups (p-values between M1+M2 and intact/M1/M2 animals: p = 0.0001/0.0004/0.0011 for mean IPI, p = 0.00001/0.0009/0.0009 for CV of IPI, p = $2X10^{-7}$/0.0003/$3X10^{-5}$ for percentage of IPIs within 15% of target).

Although M1 and M2 lesions did not abort learning, the learning rate was slower than for the control animals (Figure 5.3F). The mean number of trials required for the M1 and M2 animals to reach criterion performance (i.e., mean of IPIs was within 10% of target and CV of the IPI distribution less than 0.25) were 23,277 ± 8,485 trials (5 out of 6 animals) and 24,412 ± 12,934 trials (all 6 animals) respectively, around 50% longer than the controls (16,643 ± 7,283 trials, all 18 animals. p = 0.31

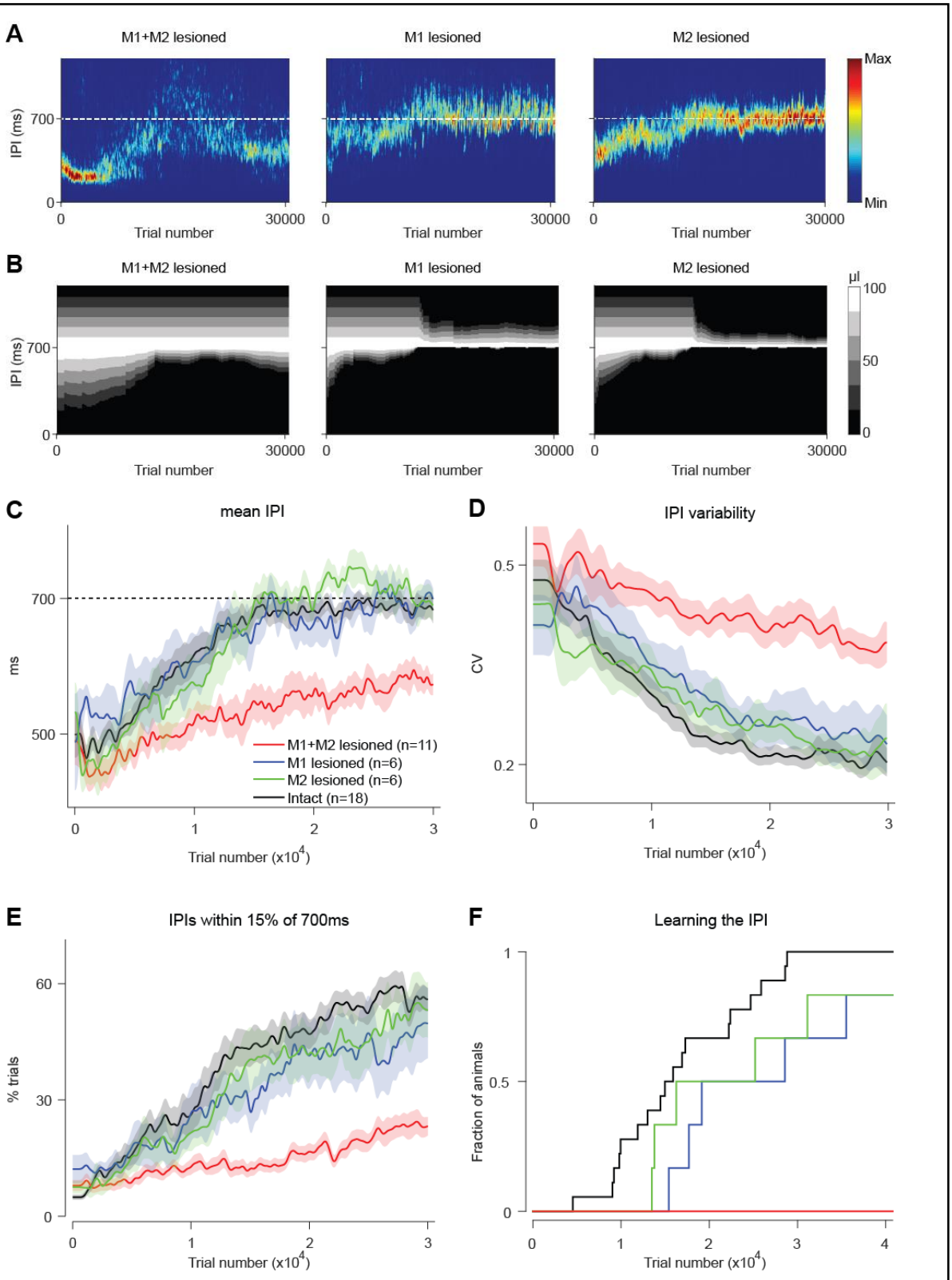

Figure 5.3 MC lesions disrupted learning, while M1 or M2 lesions slowed down but did not abort learning (see next page for details).

Figure 5.3 (continued). MC lesions disrupted learning, while M1 or M2 lesions slowed down but did not abort learning. (A) Density plots of the IPI distribution for rats with MC (left), M1 (middle), or M2 lesions (right). White dotted lines denote the 700-ms target. (B) Graded reward landscape in (A). Amount of water rewarded was automatically adjusted based on IPI to ensure a reward rate of ~35%. (C, D and E) Learning curves showing the mean IPI (C), CV of IPI (D) and percentage of trials with IPIs within 15% of target (E) as a function of training across all rats. Shaded regions denote SEM. (F) Cumulative histograms showing the fraction of animals that learned the task to criterion performance (see method) as a function of training.

between M1 and intact, p = 0.16 between M2 and intact) though not statistically significant. One M1 animal did not reach the criterion in 50,000 trials.

Furthermore, both the M1- (5 out of 6 animals) and M2- (all 6 animals) lesioned rats learned to withhold lever pressing for the predetermined 1.2s after unrewarded IPIs, whereas only 1 out of 11 MC-lesioned animals learned it (Figures 5.4A and B, see learning criteria in Method of Chapter 4). One M1 animal did not reach the criterion in 50,000 trials. After 30,000 trials of training, the percentage of unsuccessful IPIs followed by a delay of more than 1.2s was 56.3% ± 25.4% for M1 and 62.8% ± 14.7% for M2, comparable to control (61.4% ± 19.0%, p = 0.92 between M1 and intact, p = 1.00 between M2 and intact) and significantly better than M1+M2 (17.5% ± 8.4%, p = 0.0005 between M1 and M1+M2, p = 0.0001 between M2 and M1+M2). This was also reflected in the percentage of two-press trials after training (Figure 5.4C), which correlates with inter-trial delay. The percentage was 69.3% ± 9.3% for M1 and 71.2% ± 10.2% for M2, similar to control (69.3% ± 9.3%, p = 0.76 between M1 and intact, p = 0.98 between M2 and intact) and better than M1+M2 (43.8% ± 6.0%, p = 0.0011 between M1 and M1+M2, p = 2 X 10^{-5} between M2 and M1+M2). Similar to learning the IPIs, both M1 and M2 groups also showed the trend of learning the inter-trial delay more slowly than the intact group (M1: 20,232 ± 6531 trials; M2: 21,566 ± 10,135 trials; intact: 12,363 ± 7712 trials, 17 out of 18 animals. p = 0.15 between M1 and intact, p = 0.06 between M2 and intact. Figure 5.4D).

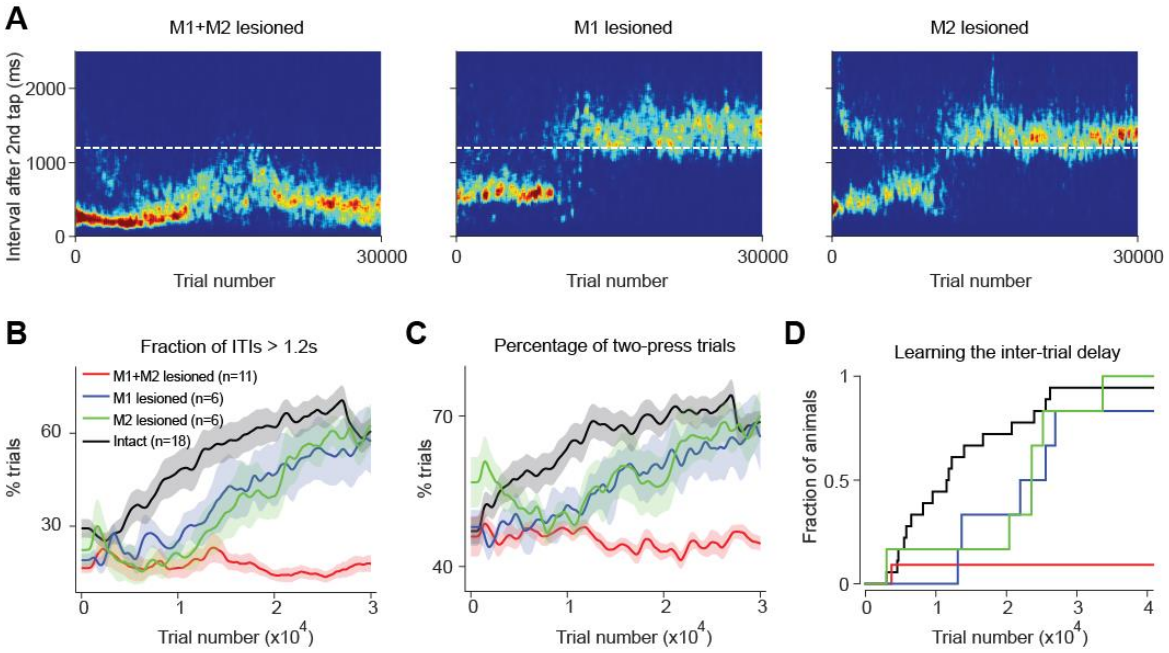

Figure 5.4 MC lesions disrupted learning of inter-trial delay, while M1 or M2 lesions did not abort learning. (A) Density plots of the delay to next lever press in rats with MC (left), M1 (middle) and M2 lesions (right). (B and C) Learning curves showing the fraction of inter-trial intervals over 1.2s (B) and percentage of two-press trials (C) as a function of training across all rats. Shaded regions denote SEM. (D) Cumulative histograms showing the fraction of animals that learned the inter-trial delay (see text for criteria) as a function of training.

Discussion

To examine the role of the M1 and M2 in learning new motor sequences, we lesioned each structure bilaterally before training rats to perform spatiotemporally precise lever-press sequences. Although motor cortex (M1+M2) lesions severely disrupted learning (Kawai et al. 2015), separate lesions of the M1 or M2 slowed down, but did not abort learning (Figures 5.2 and 5.3). The results suggest that both the M1 and M2 contribute to learning, and the two regions have, to at least a degree, redundant functions with respect to learning the task we train.

Comparison with prior M1 and M2 studies on motor learning

The MC has been implicated in dexterous motor skills in multiple studies (Alaverdashvili and Whishaw 2008; Lawrence and Hopkins 1976; Lemon 1993; Passingham, Perry, and Wilkinson 1983; Whishaw 2000), but few of them differentiated the roles of the M1 and M2. Consistent with our findings, Bailey and Mair showed that M1 or M2 lesions in rats did not abort the acquisition of nose-poke sequences guided by luminance cues, though they did affect the reaction times of the animals (Bailey and Mair 2007). On the other hand, Yin found that M2 but not M1 lesions disrupted learning of self-initiated lever-press sequences in mice (Yin 2009). Compared with our task, Yin's may have a M2-dependent cognitive component due to much longer IPIs (in the level of seconds). If so, dependency on the M1 or M2 may be related to how a sequence is trained.

Possible compensation mechanisms between the M1 and M2

Our results show that lesioning the MC severely disrupted learning of the motor task we train. However, lesioning either of its sub-components, the M1 or M2, only slowed down but did not abort learning. That means the M1 and M2 provide redundant functions and are able to cover for each other during learning. Consistent with this, the M1 and M2 have been found to compensate for each other during the recovery of dexterous movements in rhesus monkeys (Darling et al. 2009). They termed the phenomenon a "volumetric effect," similar to the classic theories of mass action (Tizard 1959; Schoenfeld and Hamilton 1977) in which the M1 and M2 are treated as one homogeneous region. Alternatively, the compensation can be explained by the overlap of M1-DLS and M2-DLS projections (Van Waes et al. 2012), implicating that the M1 and M2 have comparable capability in shaping the DLS via these projections.

A tutoring function for the MC

How exactly the MC contributes to learning complex motor sequences is still largely unknown. Since the MC is required for learning but not executing our task, we believe that the MC can re-program subcortical circuits, including the basal ganglia (Chapter 2), over weeks of training. Alternatively, the MC can also contribute to learning by facilitating subcortical plasticity or shaping motor variability (Wu et al. 2014; Chaisanguanthum, Shen, and Sabes 2014). Along these lines, the cortical area LMAN in songbirds has been found to provide error-correcting premotor biases that drive changes in downstream vocal control circuits (Andalman and Fee 2009). Currently, our lab is recording from both the MC and the DLS in behaving rats to further delineate the tutoring function of the MC.

Chapter 6 Conclusions

In this final chapter, I discuss the original contributions of the dissertation and their implications for our understanding of motor skill learning and execution. Next, I highlight the strengths and weaknesses of the study, and finally, propose directions and experiments for future research.

Implications for motor control and motor learning

In my dissertation I set out to explore the neural circuits underlying motor sequence learning and execution (Chapter 1). Specifically, my dissertation focuses on the role of the basal ganglia, a group of inter-connected subcortical nuclei, in the acquisition and execution of complex motor sequences. Eventually, I sought answers to these central questions: Is the basal ganglia circuit a critical subcortical substrate for generating complex learned motor sequences? If so, which nuclei of the basal ganglia are required for execution and learning? And finally, how are the basal ganglia tutored by the motor cortex?

The dissertation provided evidence that DLS, the sensorimotor part of the basal ganglia, is essential in complex motor sequence learning and execution. I first described a paradigm established in the lab that trains spatiotemporally precise movement sequences in rats by rewarding precise lever-press sequences (Kawai et al. 2015). While lesions of the DLS significantly disrupted performance in well-trained animals, DMS lesions had, at worst, minor effects (Chapter 2). Consistently, lesions of the GPi, a major output of the basal ganglia, acutely disrupted performance (Chapter 3). Then, by performing striatal lesions before training, I showed that the DLS is critical for acquiring task-specific motor sequences by trial and error. This suggests that the DLS is a potential subcortical target for the motor cortex's tutoring function (Chapter 4). During training, both the M1 and M2 are potentially involved as separate lesions of M1 or M2 slowed down learning (Chapter 5). Together, the chapters implicate that the sensorimotor part

of the cortical-basal ganglia circuitry is important for learning self-initiated complex motor sequences, while the basal ganglia is part of a sub-cortical motor circuit capable of autonomously executing such learned behaviors.

Previous lesion studies on the basal ganglia have largely focused on innate motor sequences that do not require any training (K. Berridge and Fentress 1987; Pellis et al. 1993; Van Den Bercken and Cools 1982). Through electrophysiological and imaging studies (Doyon et al. 2006; Grafton, Hazeltine, and Ivry 1995; Rauch et al. 2004; Rauch et al. 1998; Barnes et al. 2005; Costa, Cohen, and Nicolelis 2004) have demonstrated a correlation between neural activity in the basal ganglia and motor sequence execution (Henson 2005; Paus 2005; Brown and Eyler 2006; Weber and Thompson-Schill 2010; Humphrey 2000; Sporns 2010); in most cases, causality has not been demonstrated. By lesioning the striatum and GPi, I provide direct evidence supporting the importance of the basal ganglia in executing learned motor sequences.

Furthermore, this dissertation informs our view of the neural circuits involved in different types of motor sequence learning. Learning our self-initiated lever-pressing task requires both the MC and DLS, the sensorimotor portion of the cortical-basal ganglia circuitry, but not the DMS. In contrast, most primate studies use visuomotor tasks that involve externally guided movements, and these have implicated the associative portion of the circuitry, including the DMS and the prefrontal cortex, in learning (Miyachi et al. 1997; Okihide Hikosaka et al. 2002). Even though both tasks require sequencing of forelimb movements, the brain regions recruited during learning differ. A tentative conclusion from comparing these studies is that learning processes relying heavily on external cues or instructions will depend more on the associative loop (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995). On the other hand, our self-initiated task focuses on achieving spatiotemporal precision through a process of trial-and-error, which seems to depend preferentially on sensorimotor loops for learning.

Finally, our systematic lesion studies pave the way for understanding the neural circuits underlying learned motor sequences. This high-throughput study was made possible by an automated rodent training system (Poddar, Kawai, and Ölveczky 2013). Importantly, these animals were trained for the exact same motor task under the same procedure, making fair comparisons between lesion groups possible. While this dissertation emphasizes the cortical-basal ganglia circuits, further studies extending to other brain regions would reveal a more complete picture of the circuitry underlying learned motor sequences.

Strengths and weaknesses of the dissertation

The main behavioral paradigm used in Chapters 2 through 5 is the timed, self-initiated lever-pressing task (Chapter 2 Method). Compared to with other rodent lever-pressing tasks (such as Yin 2010), one major strength is that it produces spatiotemporally precise motor sequences. After training, the asymptotic mean IPI was 704ms ± 24ms with a CV of 0.18 ± 0.03 (Chapter 2). Such a low temporal variation across trials and across animals allows easier observation of statistically significant changes in motor performance before and after lesions. Moreover, since the high-throughput training was fully automated under a standardized procedure, we minimized biases introduced by human intervention (Hurst and West 2010; Sorge et al. 2014). All of this improved the credibility of our findings.

Another main method used in this dissertation is acute brain lesion. Pharmacological lesions were performed to establish causal links between brain regions and motor functions. Although excitotoxins spare axons from non-targeted brain regions (Schwarcz, Whetsell, and Mangano 1983; Coyle 1981), the spread of the drug molecules within brain tissues can never be perfectly controlled, leading to variability in lesion volume. However, if a brain region is necessary for performance of the motor task, one would expect a correlation between lesion volume and performance degradation. Therefore, I quantified the

lesion volume of the DLS in Chapters 2 and 4, and I showed that stronger deficits in performance could be caused by removing a larger proportion of the region. That analysis also ruled out the possibility that deficits were caused by random secondary effects in other brain regions (Schoenfeld and Hamilton 1977; Finger, Walbran, and Stein 1973; Nudo 1999).

Recommendation for future directions

This dissertation suggests that the sensorimotor part of the cortical-basal ganglia circuitry is critical for learning and executing the motor skill we trained. Therefore, one major follow-up question directly related to the theme is: How do the MC and the basal ganglia collaborate to learn and/or generate motor sequences?

In Chapter 4, I argue that the DLS is possibly a direct target for the MC's tutoring given the anatomical and behavioral evidence. Though it is likely, it still must be proven. One possible experiment is to silence or disrupt the MC-DLS connections by genetic means and assess the effect this has on motor learning. However, the experiment's drawback is that the efficacy and reliability of silencing depends on the transfection efficiency and toxicity of the viral particles. The experiment will become more feasible when genetic tools continue to improve.

Another informative experiment would be to optogenetically identify and record from the MC neurons projecting to the DLS (Cohen et al. 2012). The projection neurons can be tagged by light-sensitive channelrhodopsin and identified by their responses to optical stimulation. Analyzing the neural activity of these projections during learning will help to identify the tutoring signals, if there are any.

Also, to figure out how the DLS contributes to sequence acquisition, we can characterize changes in the neural representations of motor sequences in the DLS during learning. Chronic neural recordings

can be performed when the animals are working on the lever-pressing task. For instance, as the motor sequence gradually consolidates, the neural activity of DLS neurons may represent individual motor elements, or only the start/end of the sequence. The former implicates that the basal ganglia continue to serially select motor elements, while the latter suggests that the basal ganglia simply select and initiate a consolidated sequence after training.

A second major follow-up question of this dissertation involves completion of the neural circuitry for the motor skill. We found that learning requires the MC and DLS, but this does not necessarily mean that other brain regions are not involved. Anatomical studies have suggested that the basal ganglia form cortical loops (Alexander, DeLong, and Strick 1986; Middleton and Strick 2000; Parent and Hazrati 1995) and subcortical loops (Mchaffie et al. 2005; Nandi et al. 2002; Redgrave and Coizet 2007) with the thalamus. Since the architecture of loops likely provides computational solutions for action selection and reinforcement learning (Hikosaka et al. 2002; Ito and Doya 2011), the thalamus is an attractive candidate for future studies on motor learning and execution.

Furthermore, this dissertation does not distinguish between the motor functions of the direct and indirect pathways of the basal ganglia. Investigating the STN and GPe of the indirect pathway will shed light on that problem.

To summarize, the DLS but not the DMS is required for both learning and executing a spatiotemporally precise sequence without external cues. During learning, the DLS is possibly tutored by the MC, including M1 and M2. After learning, the MC is no longer required for execution.

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